C S I R

INVESTIGATIONS

COMPOSITION AND NUTRITIVE VALUE OF VANASPATI

INVESTIGATIONS ON

THE COMPOSITION AND NUTRITIVE VALUE OF VANASPATI

being

reports of researches conducted under the auspices of the Ministry of Food and Agriculture and the Council of Scientific & Industrial Research

THE COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH
NEW DELHI
1952

PREFACE

The investigations described in the following pages deal with different aspects of the vanaspati enquiry undertaken under the joint auspices of the Ministry of Food & Agriculture of the Government of India and the Council of Scientific & Industrial Research.

With the concurrence of the Ministry of Food & Agriculture, the publication of the work was undertaken by the Council of Scientific & Industrial Research, which entrusted the work to a Committee consisting of Drs. V. Subrahmanayan (convener), B. C. Guha and D. V. Karmarkar. The material was collected during 1951, but it took some months to check up the data and edit the manuscripts.

The thanks of the Committee are due to the contributors, the Ministry of Food & Agriculture and the Council of Scientific & Industrial Research who placed all the material at the disposal of the Committee. The Committee also wishes to place on record its grateful thanks to Dr. S. S. Bhatnagar, F. R.S., Director, Scientific & Industrial Research, and Secretary, Ministry of Natural Resources and Scientific Research, for encouragement and support, and to Dr. S. M. Bose of the Central Food Technological Research Institute, Mysore, for his assistance in bringing the materials into a form suitable for publication.

V. SUBRAHMANYAN

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CONTENTS

PART I

PART II

PART 1

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INTRODUCTION

The vanaspati* industry in India is of comparatively recent origin. Production was first started on a small scale in 1930. Prior to that year, however, vanaspati was being imported from Europe. It gradually gained in popularity and production increased from year to year. During World War II, there was considerable demand for vanaspati from both the Defence Services and the civilian population, production rose steadily from 65,000 tons in 1940 to 134,000 tons in 1945, and the manufacture of vanaspati became one of the major food industries of the country.

The Government of India set up an Advisory Committee to consider the expansion of the vanaspati industry in India. On the recommendation of the Committee, a scheme for a three-fold development of the industry was approved by the Food Department in 1945. Sanction was accorded to the establishment of 50 or more factories in different parts of the country, thus bringing the total production capacity to 450,000 tons per year.

There was, however, a considerable volume of opposition to the proposed expansion. The ghee manufacturers felt that the increase in vanaspati production would affect the interests of the ghee industry and the cattle breeding industry in general. The nutritive value of vanaspati was also questioned. An important section of consumers and popular leaders held the opinion that vanaspati was injurious to health and that its production should be discouraged, if not stopped altogether. Ray and Pal[1] of the Indian Veterinary Research Institute, Izatnagar, reported that vanaspati, fed to rats at a sub-optimal level of nutrition, affected the growth of the second and third generations. Evidence of poor storage of vitamin A in the liver, as also of symptoms of vitamin B-deficiency consequent on prolonged feeding with vanaspati as the main source of fat, were also reported by the same authors. These results led to misgivings about the use of vanaspati as an article of human food.

The results reported by Ray and Pal were not in accordance with those reported by other scientific workers, both in India and abroad. The nutritive value of vanaspati was taken up for discussion at a meeting of the Technical Panel of the then Department of Food, held at Delhi on 26 November, 1946. In his address to the Panel, the Hon'ble Dr. Rajendra Prasad, then the Hon'ble Member for Food and Agriculture, urged that a comprehensive and critical scientific investigation of the subject should be undertaken to obtain definite and conclusive

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* The term vanaspati is the popular name for refined, hardened (hydrogenated) and deodorised vegetable oil used for cooking in India. It corresponds to shortening used in other parts of the world. Groundnut (peanut) oil is chiefly used in the manufacture of the product.

[1]Ray & Pal, *Sci. & Cult.*, **12** (1947), 494.

evidence on the basis of which Government could take a decision in regard to the future of the industry. Acting on this suggestion, the Technical Panel formed a Vanaspati Research Planning Committee of scientific workers, representing different laboratories interested in food and nutrition problems, to plan and carry out coordinated experiments at a number of centres and to present a precise and authoritative report on the subject. The following were the members of the Committee :

Dr. K. P. Basu, Dairy Chemist, Indian Dairy Research Institute, Bangalore ; Dr. M. Damodaran, formerly Professor of Biochemistry, University of Madras, and now Assistant Director, Division of Biochemistry, National Chemical Laboratory of India, Poona ; Dr. B. C. Guha, formerly Chief Technical Adviser to the Food Department, New Delhi, and now Member, Damodar Valley Corporation, Calcutta ; Dr. D. V. Karmarkar, Technical Adviser, Ministry of Food and Agriculture, New Delhi ; Dr. N. D. Kehar, Officer-in-Charge, Animal Nutrition Section, Indian Veterinary Research Institute, Izatnagar ; Lt.-Col. C. K. Lakshmanan, Director, All-India Institute of Hygiene and Public Health, Calcutta ; Dr. K. Mitra, formerly Nutrition Adviser and now Assistant Director-General of Health Services, Ministry of Health, New Delhi ; Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Indian Council of Medical Research, Coonoor ; Dr. M. V. Radhakrishna Rao, Assistant Director, Haffkine Institute and Nutrition Officer, Government of Bombay, Bombay ; Dr. K. Rajagopal, formerly Associate Professor and now Professor of Biochemistry and Nutrition, All-India Institute of Hygiene and Public Health, Calcutta ; Dr. G. Sankaran, formerly Professor of Biochemistry and Nutrition, All-India Institute of Hygiene and Public Health, Calcutta, and now Member, Indian Penicillin Committee, Bombay ; Maj.-Gen. S. S. Sokhey, Director, Haffkine Institute, Parel, Bombay ; Dr. V. Subrahmanyan, formerly Professor of Biochemistry, Indian Institute of Science, Bangalore and now Director, Central Food Technological Research Institute, Mysore ; Mr. S. H. Turner, Factory Manager, Hindustan Vanaspati Manufacturing Co. Ltd., Bombay ; and Lt.-Col. O. P. Verma, Officer Commanding, Central Composite Food Laboratory, Army Headquarters, Q. M. G.'s Branch, New Delhi.

Considerable amount of work has been done in Europe and America on the relative nutritive values of vegetable oils, hydrogenated fats and butterfat. Most of these studies, however, have been carried out with experimental animals receiving adequate diets. The majority of the people of India live on poor cereal diets which are characterised by deficiency in most of the essential constituents. It was considered, therefore, to be of practical interest to investigate the role of vanaspati as compared with other fats in animal and human nutrition using the poor rice diet of South India, which is deficient in proteins, minerals and vitamins.

At their first meeting held on 28 January, 1947, the Vanaspati Research Planning Committee drew up a programme of studies using both experimental animals and human subjects with special reference to the conditions prevalent in India. The experiments were divided into 3 parts: (1) Animal experiments (2) Human metabolism studies (3) Institution feeding experiments.

The animal experiments were designed to study the effect of vanaspati as compared with the corresponding raw and refined oils and also with

ghee, when fed at 5 per cent level*. Five fats, viz., (1) raw groundnut oil, (2) refined groundnut oil as prepared out of (1), (3) vanaspati of m.p. 37°C. and (4) vanaspati of m.p. 41°C., both prepared out of (2), and (5) cow ghee as prepared from dairy butter, were compared using different basal diets, viz., (1) synthetic diet adequate in regard to protein, minerals and vitamins, (2) poor rice diet, (3) poor rice diet supplemented with vitamins, (4) poor rice diet supplemented with casein, (5) poor rice diet supplemented with calcium, and (6) poor Bengali diet. Apart from growth measurements and studies on breeding and lactating capacity of rats, metabolism studies on the utilization of fat, protein and minerals were carried out in the different series of experiments. The storage of vitamin A in livers was also studied on the completion of each series of experiments. In most cases, experiments were continued for 3 generations.

Human metabolism studies were carried out with adult human subjects receiving oil, vanaspati or ghee as the main source of fat. As in the case of animal experiments, the poor rice diet formed the basal diet for these studies and the same 5 fats were compared. Apart from fat metabolism, the influence of ingested fats on the utilization of dietary protein and minerals was also studied.

The institution feeding experiments were conducted with children under 15 years of age receiving a predominantly poor cereal diet supplemented with about 5 per cent fat in the form of either raw groundnut oil or vanaspati of m.p. 37°C. In this case, the comparison was between raw oil and vanaspati only, the main object being to study whether any characteristic clinical symptom which was not observed in the case of oil was noticeable in the case of vanaspati. Metabolism studies also were carried out with children.

The animal experiments were carried out at the following centres. The names of Members of the Committee who supervised the work are also given below:

(1) Indian Dairy Research Institute, Bangalore.
(Dr. K. P. Basu).

(2) Indian Institute of Science, Bangalore.
(Dr. V. Subrahmanyan).

(3) Indian Veterinary Research Institute, Izatnagar.
(Dr. N. D. Kehar).

(4) University College of Science and Technology, Calcutta.
(Dr. B. C. Guha).

The human metabolism experiments were conducted at:

(1) Indian Institute of Science, Bangalore.
(Dr. V. Subrahmanyan).

(2) Nutrition Research Laboratories, Coonoor.
(Dr. V. N. Patwardhan).

*Diet surveys in India have shown that the fat content of poor rice diets is low and usually does not exceed 5 per cent. In the experiments with poor rice diets, therefore, 5 per cent fat level was suggested.

The institution feeding experiments were arranged at:

(1) Aryan Orphanage, Daryaganj, Delhi.
(Drs. K. Mitra and D. V. Karmarkar).

(2) David Sassoon Industrial School, Bombay.
(Dr. M. V. Radhakrishna Rao).

(3) St. Philomena's Orphanage and Good Shepherd Convent, Mysore.
(Dr. V. Subrahmanyan).

Interim reports from the different centres were submitted to the Vanaspati Research Planning Committee which held meetings from time to time, when the progress of the work was discussed and necessary amendments made in the programme of work. By the time of the fifth meeting of the Committee, held on 24 November, 1949, all the experimental work was completed and final reports from different centres were available. Based on the results obtained by the different laboratories, the Committee came to the following main conclusion:

> "In comparative feeding experiments carried out at 4 different research centres on rats for 3 generations with raw groundnut oil, refined groundnut oil and vanaspati of melting points 37°C. and 41°C., the results indicate that there is no deleterious effect produced by vanaspati as compared with raw or refined oil."

Further, in the light of the results obtained on short term human metabolism experiments conducted in 2 laboratories and long term human feeding experiments carried out at different orphanages, the Committee came to the following conclusion:

> "Human feeding trials carried out at 4 different centres also indicate that vanaspati of melting point 37°C. has no harmful effect as compared with raw groundnut oil."

While the above conclusions were reached, it was considered necessary by the Committee to examine statistically the results obtained in the different series of experiments in order to find out finer differences, if any, in the relative nutritive values of the different oils and fats which were ingested along with the different diets.

The statistical analysis of the data obtained in the experiments with rats as well as with human subjects was carried out at the All-India Institute of Hygiene & Public Health, Calcutta, under the supervision of Prof. S. Swaroop. The statistical report was considered by the Committee at their sixth meeting held on 25 August, 1950. The main conclusions emerging out of the statistical analysis were summarised and the following opinion was expressed by the Committee as regards the nutritive value of different oils and fats:

> "Feeding experiments with poor rice diets* carried out on rats as well as on human subjects at different centres of research

*Dr. N. D. Kehar was of the opinion that "synthetic diet" should be included along with "poor rice diets".

have not shown vanaspati of melting point 37°C. to have any deleterious effect as compared with raw and refined groundnut oil. It appears that vanaspati of melting point 41°C. is absorbed to a lesser extent than raw groundnut oil and that it may have an adverse effect on calcium utilization, although definite conclusions cannot be drawn from the limited series of experiments on calcium metabolism. As regards comparative nutritive values of (1) pure ghee, (2) raw groundnut oil, (3) refined groundnut oil, (4) vanaspati of melting point 37°C. and (5) vanaspati of melting point 41°C., the balance of experimental evidence places ghee as the best ; raw groundnut oil, refined groundnut oil and vanaspati of melting point 37°C. fall into one group and are next best to pure ghee ; vanaspati of melting point 41°C. comes third in nutritive value."

The enquiry into the nutritive value of vanaspati represents the concerted effort on the part of several institutions distributed all over India. Over 25 research workers participated in the research. This collaborative effort dealing with such an important subject was rendered possible at the initiative of the Government of India through the Ministry of Food and later through the Ministry of Food and Agriculture.

In India the investigations on the comparative nutritive value of ghee, vegetable oils and vanaspati were first started in April 1944 under the auspices of the Indian Council of Agricultural Research at the Animal Nutrition Division, Indian Veterinary Research Institute, Izatnagar, U.P. and at the Indian Dairy Research Institute, Bangalore. At the Indian Dairy Research Institute work was carried out for about 18 months, while the work at the Indian Veterinary Research Institute was periodically extended and covered a period of six years. The investigations at the Indian Veterinary Research Institute embraced a wider range of problems. The consolidated results of the experiments at Indian Veterinary Research Institute were examined and adopted at a joint meeting of the Animal Nutrition Committee of the Indian Council of Agricultural Research and the Vanaspati Research Advisory Committee of the Council of Scientific and Industrial Research. While certain results obtained at Indian Dairy Research Institute were published in Indian Journal of Veterinary Science and Animal Husbandry, the Indian Council of Agricultural Research are publishing the findings obtained under the two schemes in a separate monograph.

ANIMAL EXPERIMENTS

Growth experiments on rats have been extensively carried out to study the relative nutritive values of various dietary fats. There are several conflicting reports concerning the nutritive value of margarine as compared to that of butterfat. Hoagland and Snider[1] found that the average growth rates of rats for different lards were essentially the same as the average rates for shortenings. Harris and Mosher[2], Henry *et al.*[3] and Parish *et al.*[4] reported that it is unlikely that butterfat possesses nutritive properties superior to those of the vegetable oils studied, viz., peanut, cottonseed, soyabean and corn oils. Euler *et al.*[5,6] concluded that when adequately fortified with vitamins, margarine is not inferior to butter in its growth promoting qualities. Deuel *et al.* [7,8] found no difference in the growth of weanling rats fed on an adequate diet in which the fat component was butter, margarine, olive, groundnut, maize, cottonseed or soyabean oil. Deuel *et al.*[9] studied reproduction and growth rates of rats for over 10 generations on an adequate diet and concluded that margarine could adequately replace butterfat. Deuel *et al.*[10] compared growths of rats on restricted and adequate calorie intakes and also on injection of growth hormone and observed no difference in nutritive value between butter, margarine, a commercial hydrogenated fat, corn, cottonseed, peanut or soyabean oil. Deuel[11] has summarised the scientific evidence which led him to conclude that the fats in butter and margarine have essentially the same nutritive value. Adequacy of margarine to meet the dietary needs for fat was indicated in Deuel's report[12], according to which rats fed on a diet consisting only of skimmed milk powder, margarine fat, ground whole wheat and sodium chloride progressed to the twentyfifth generation without indication of an approaching deficiency.

On the other hand, Schantz *et al.*[13,14] and Boutwell *et al.*[15] found that when lactose was the sole carbohydrate in the diet, butterfat was slightly superior to margarine and various vegetable oils. Boutwell *et al.*[16] found that with a mixed carbohydrate diet, vitamin-fortified oleomargarine produced a growth response equal to that of butterfat ; however, with lactose as the sole carbohydrate, margarine was inferior. Boer[17] postulated from his rat growth studies the presence of a new growth factor in butter acids. Boer and Jansen[18] reported that for promoting growth, butterfat is superior to margarine, peanut, olive and other vegetable oils even though the latter group of fats were fortified with vitamins A and D. Boer *et al.*[19] reported that growth with summer butterfat was slightly but significantly greater than that with groundnut, olive and rapeseed oils. The substance responsible for the superior growth was traced to vaccenic acid. They[20] also observed a significant difference in growth promoting properties between rapeseed oil alone and rapeseed oil containing crude preparations of vaccenic acid. Later, however, Boer *et al.*[21] stated that spectroscopically-pure vaccenic acid has no special nutritive property and concluded that the growth promoting properties of summer butter may be due to other trace materials. The recent reports of Deuel *et al.*[22], Natta *et al.*[23] and Euler *et al.*[24] also establish that vaccenic acid

has no special growth stimulating action in rats. Euler *et al.*[25] found that margarine promotes better growth than butterfat. Euler *et al.*[26] also presented evidence to show that certain margarine fats may be even superior to butterfat when evaluated on the basis of reproduction and lactation.

The relative nutritive merits of raw and hydrogenated vegetable oils on the one hand and melted butterfat (ghee) on the other, were investigated by Ray and Pal[27] at the Indian Veterinary Research Institute, Izatnagar. These investigators reported that the growth rates of rats fed on a sub-optimal diet *plus* hydrogenated vegetable oils (vanaspati) were significantly lower than those of rats fed on the same diet *plus* ghee in the second and third generations and that absorption and utilization of carotene were affected when rats were fed on vanaspati diet. They also reported that extensive alopecia was seen in animals of the vanaspati group, especially in the second generation, whereas ghee-fed animals were not affected. In another series of experiments using chicks as experimental animals, the same authors reported that symptoms of vitamin B-deficiency, cutled-toe paralysis, dermatitis, and poor growth, were observed in animals fed on a diet containing vanaspati, whereas those receiving ghee remained immune. In the experiments carried out at the Indian Dairy Research Institute, Bangalore, however, no significant differences in the growth promoting values of oil, vanaspati or ghee were observed and no pathological symptoms developed in experimental animals.

The Vanaspati Research Planning Committee, appointed by the Government of India in 1947, undertook a comprehensive inquiry into the nutritive merits of vanaspati with a view to find an answer to the much disputed question whether vanaspati was deleterious to health. The cooperation of a number of research laboratories in India was secured and an identical plan of investigation was recommended for adoption at different centres, using experimental materials supplied, as far as possible, from a central source. These steps were necessary in order to ensure that the results obtained in different laboratories were strictly comparable.

The experimental diets, according to the plan recommended by the Vanaspati Research Planning Committee, were the following:

Series I Synthetic diet
Series II Poor rice diet
Series III Poor rice diet + yeast and vitamins A, D and E
Series IV Poor rice diet + calcium carbonate
Series V Poor rice diet + casein
Series VI Poor Bengali diet

The experimental animals under each series were divided into 5 groups, each group receiving one of the following 5 fats:

Group 1 Cow ghee
Group 2 Raw groundnut (G. N.) oil
Group 3 Refined groundnut (G. N.) oil
Group 4 Vanaspati of m.p., 37°C.
Group 5 Vanaspati of m.p., 41°C.

The percentage compositions of the different diets were as follows :

(1) *Synthetic diet*—Maize starch, 55 ; extracted casein, 15; cane sugar, 10 ; salt mixture (Osborne and Mendel), 5 ; yeast powder, 5 ; and oil or fat (to be tested) 10°/₀ ; vitamin A, 60 I. U. per rat per day ; vitamin D, 10 I. U. per rat per day ; and vitamin E, 0.5 mg. ∝-tocopherol acetate per rat per day.

(2) *Poor rice diet*—Polished rice, 78.5, tur or arhar dal (*Cajanus cajan*), 5.0 ; non-leafy vegetables — potatoes and brinjals (*Solanum melongena*), 8.2 ; leafy vegetables (*Amaranthus gangeticus*), 2.1 ; whole milk powder KLIM), 0.9 ; common salt, 0.3 and oil or fat (to be tested), 5.0°/₀.

As the experimental animals showed poor growth on the above diet and as it is known that tamarind (*Tamarindus indicus*) forms an essential part of the South Indian diet, it was included in subsequent experiments. A small percentage of calcium carbonate was also included to facilitate better growth in animals. The composition of the modified poor rice diet was as follows :

(3) *Modified poor rice diet* :—Polished rice, 75.1 ; tur-dal, 5.0 ; non-leafy vegetables, 8.2 ; leafy vegetables, 4.2 ; whole milk powder, 0.9 ; common salt, 0.5 ; tamarind (whole ripe), 1.0 ; calcium carbonate, 0.1 and oil or fat (to be tested), 5.0°/₀.

(4) *Supplemented poor rice diets*—

(*a*) *Vitamin supplement series*—Yeast (Squibb's) 4.0°/₀ of the diet; vitamin A, 60 I. U. per rat per day; vitamin D, 10 I. U. per rat per day ; vitamin E, 0.5 mg. ∝-tocopherol acetate per rat per day.

(*b*) *Calcium carbonate supplement series*—Calcium carbonate 0.3 of the diet.

(*c*) *Casein supplement series*—Casein 7.0°/₀ of the diet.

In the above 3 series of supplemented diets, the supplements replaced the same quantity of polished rice in the diet.

(5) *Poor Bengali diet A*—Pelished rice, 65.5 ; masur dal, (*Lens esculenta*) 2.0 and mung dal, (*Phaseolus radiatus*) 0.5 ; leafy vegetables, 1.2 ; — potatoes, 11.0; brinjals, 4.5 ; torai (*Luffa acutangula*), 4.5 ; onions, 2.0 ; fish, 2.0 ; egg, 1.0 ; meat, 0.5 ; common salt, 0.3 and oil or fat (to be tested), 5.0°/₀.

The composition of the poor Bengali diet was subsequently modified as follows :

(6) *Poor Bengali diet B*—Polished rice, 74.0 ; tur or arhar dal, 1.2 ; leafy vegetables, 0.6 ; potatoes, 10.0 ; brinjals, 5.0 ; torai, 5.0 ; fish, 2.0 ; mustard oil, 1.0 ; salt, 0.3. 95 parts of the diet were mixed with 5 parts of the oil or fat (to be tested).

The experiments were carried out for 3 generations. Besides noting the rates of growth, food intake and ability for reproduction and lactation,

the conditions of the skin, fur, eyes, tail and paws were kept under observation. The observations were specially directed to determine whether there was any evidence of the following :

(1) Skin, tail and paws — undue dryness and open lesions like ulcers
(2) Hair — rough coat, shedding of hair, if any, and the extent of the same
(3) Eyes — dryness, opacity and ulcers, if any

Vitamin A and fat contents of the liver of experimental animals were also estimated.

Samples of raw and refined groundnut oils and 2 vanaspatis of m.p. 37°C. and 41°C. were obtained from the Hindustan Vanaspati Manufacturing Co., Ltd., Bombay ; cow ghee from the Indian Dairy Research Institute, Bangalore ; maize starch (Harvest Queen Brand) from the Corn Products Co. Ltd., Bombay ; extracted casein from the Indian Institute of Science, Bangalore ; and vitamins A, D and E and propylene glycol from the Haffkine Institute, Bombay. Rice and pulses were supplied by the Ministry of Food, Government of India, New Delhi.

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SECTION I

1. Animal Experiments carried out at the Indian Dairy Research Institute, Bangalore*

The following series of experiments were conducted:

Series I Synthetic diet
Series II Poor rice diet
Series III Poor rice diet + yeast + vitamins A, D and E
Series IV Poor rice diet + calcium carbonate
Series V Poor rice diet + casein
Series VI Poor Bengali diet A
Series VII Poor Bengali diet B (cooked)
Series VIII Poor Bengali diet B (uncooked)

In each series of experiments, one group of rats was kept on the basal diet alone without any fat for the sake of comparison.

PREPARATION OF DIETS AND FEEDING

A weighed amount of the daily ration *minus* the fat was taken, mixed with the requisite amount of fat (first heated to 250°C. for 15 minutes and then cooled), and water to make a paste and cooked in a vessel of boiling water. Cooked food was weighed and given to each rat in such quantity that a little was left over every day. The remains were collected daily, dried and weighed once a week.

All growth experiments were continued for 13 weeks, the first week being considered as the preliminary period. The rats were then mated.

In the selection of animals for experimental purposes, young albino rats, 28 days old (wt., 35-40 g.), were weaned and divided into 6 groups, each of 12 animals (6 males and 6 females). Care was taken to distribute litter mates as far as possible equally among the different groups. Each group of animals represented one of the 5 fat groups; the sixth group fed on the diet without fat served as the control. The animals were kept in numbered cages. The total number of animals required for each series of experiments was 6×12 or 72.

The animals were kept under observation for 3 months. They were weighed once a week, and any symptom of a pathological nature noted.

After the expiry of the experimental period of 3 months, the rats were mated among the same group. Neither the body weight nor the food left over was recorded during the period. Only reproductive ability and lactating capacity were noted.

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*The work described in this section was carried out under the supervision of Dr. K. P. Basu, Dairy Chemist, Indian Dairy Research Institute, Bangalore.

The experimental work was mostly carried out by Shri Arun Sen Gupta, Research Assistant, who worked on the scheme throughout its duration.

Shri S. R. Jayashankar, Research Assistant, joined the scheme about a year after the scheme had started.

Shri Byroji Rao, Supervisor, assisted in the maintenance and care of experimental animals.

The young ones born in the second and the third generations were maintained on the same diet schedule after they attained the required initial weights. Feeding trials could not be started on the twentyeighth day as almost always young rats were very much under-weight. They were fed with experimental diets supplemented with 1 cc. of whole milk till they attained an average weight of 20 g., when they were put on the experimental diet.

EXPERIMENTAL RESULTS

Series I, Synthetic Diet

First Generation

Experiments on the growth of rats of the first generation fed on a complete synthetic diet containing various fats were carried out. The growth of animals in this series of experiments was somewhat comparable to that obtained with the stock diet. During the experimental period of 12 weeks, only 1 or 2 rats suffered slightly from alopecia but soon recovered. Table 1 gives the average weekly growth *of experimental animals of the first generation on this diet.

Table 1: Average weekly increase in weights (in g.) of experimental animals — Series I, first generation

Group	Male	Female
Ghee	10.60	6.25
Raw G. N. oil	10.00	6.00
Refined G. N oil	10.42	5.84
Vanaspati, m. p., 37°C.	9.60	5.44
Vanaspati, m. p., 41°C.	10.10	5.50
Basal diet – fat	9.83	6.00
Stock diet	12.90	7.16

There were hardly any symptoms of a pathological nature among the animals during the experimental period. All the rats survived the experimental period.

The reproductive and lactating capacities of female rats in all the groups were poor. Many of the animals, both male and female, showed signs of deficiency, weakness of hind limbs and slight bleeding through the nose and the paws. The symptoms were probably due to the lack of vitamin E. Some of the female rats proved sterile and a few died in pregnancy. There were 1 or 2 cases of abortion. Most of the animals conceived late. The animals in the basal diet gruop without fat and those in the refined oil group were sterile. Table 2 gives the reproductive and lactating capacities of animals of the first generation.

*In this and the subsequent Tables, only the average figures have been given. The data for individual animals were utilised for statistical analyses.

Table 2: Reproductive and lactating capacities of experimental animals— Series I, first generation

Group	No. of females	No. sterile	No. died in pregnancy	No. young born	No. surviving
Ghee	6	1	..	27	4
Raw G. N. oil ..	6	2	..	22	8
Refined G. N. oil ..	6	4	2	..	..
Vanaspati, m. p., 37°C.	6	..	1	25	3
Vanaspati, m. p., 41° C.	6	..	..	33	9
Basal diet – fat ..	6	5	1	..	..

Second Generation

The animals of the second generation were weak and lethargic with scanty fur coats and were under-weight (12-14 g.) at weaning time. Only 24 young ones were available for the second generation experiment. They were kept on the experimental diet supplemented with 5 cc. of skimmed milk till they attained a weight of 20 g. They were then fed on the experimental diet. Their condition improved ; they soon gained in weight and were restored to a fairly good state of health in a short period. The rate of growth was good though somewhat less than that of rats of the first generation. In general, the second generation of rats showed better growth and better state of health when fed on this diet in comparison with the growth and health of the corresponding generation of rats on other diets.

Table 3: Average weekly increase in weights (in g.) of experimental animals—Series I, second generation

Group	Male	Female
Ghee	9.00	4.25
Raw G. N. oil	8.50	4.67
Refined G. N. oil	..	..
Vanaspati, m. p., 37°C. ..	6.20	4.60
Vanaspati, m. p., 41°C. ..	6.83	5.25
Basal diet – fat	..	..

The pathological symptoms observed were : one case of lesion, a centimeter long on the right shoulder blade of a rat of the ghee group. There was no case of alopecia.

No symptoms of bleeding or weakness of hind limbs were observed. Table 4 gives the reproductive ability of the second generation animals. Only one rat conceived but the young ones born were killed by the mother rat. The experiment could not, therefore, be extended up to the third generation.

Table 4: Reproductive and lactating capacities of experimental animals—Series I, second generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee	2	1	2	..
Raw G. N. oil ..	5	5	..	..
Refined G. N. oil ..	..	..	..	..
Vanaspati, m.p., 37°C.	1	1	..	..
Vanaspati, m. p., 41°C.	6	6	..	..
Basal diet—Fat ..	..	..	..	..

Series II Poor Rice Diet

First Generation

The growth was found to be uniformly poor, irrespective of the nature of the fat given. Table 5 gives the average weekly weights of animals fed on the diet.

Table 5: Average weekly increase in weights (in g.) of experimental animals—Series II, first generation

Group	Male	Female
Ghee	4.52	2.90
Raw G. N. oil	3.14	2.97
Refined G. N. oil	4.13	3.20
Vanaspati, m. p., 37°C. ..	3.70	2.96
Vanaspati, m. p., 41°C. ..	4.03	3.36
Basal diet—fat ..	4.10	2.90

Some of the rats (*c.* 2 in each group) in all the groups of the first generation suffered from alopecia. No group, except the diet without fat group, was free from these symptoms. Two animals belonging to the raw groundnut oil group and 2 from the refined oil group died during the experimental period.

A large number of the animals proved sterile ; only one litter of 6 animals belonging to the vanaspati of m.p., 41°C. group survived. Table 6 gives the reproductive and lactating capacities of animals of the first generation.

Table 6: Reproductive and lactating capacities of experimental animals—Series II, first generation

Group	No. of females	No. sterile	No. died in pregnancy	No. young born	No. surviving
Ghee	6	1	..	24	..
Raw G. N. oil ..	6	1	1	14	..
Refined G. N. oil	5	2	..	12	..
Vanaspati, m. p., 37°C.	6	1	2	18	..
Vanaspati, m. p., 41°C.	6	3	..	16	6
Basal diet—fat ..	6	4	..	10	..

Second Generation

The 6 second generation animals (3 males and 3 females), all belonging to the vanaspati, m.p., 41°C. group, were under-weight (8-12 g.) on the twenty-first day. They were put on the experimental diet after feeding them on a diet containing a supplement of 1 cc. whole milk until they attained the average weight of 20 g. The growth of the animals was poor and one of the female rats died during the experimental period. Table 7 gives the average growth of animals.

Table 7: Average weekly increase in weight (in g.) of experimental animals—Series II, second generation

Group	Male	Female
Vanaspati, m. p., 41°C. ..	3.00	2.25

SERIES III: POOR RICE DIET SUPPLEMENTED WITH YEAST VITAMINS

First Generation

In this series of experiments, the growth of animals was much better than that of animals of Series II. The average weekly weight increments of male and female rats are given in Table 8.

Table 8: Average weekly increase in weight (in g.) of experimental animals—Series III, first generation

Group	Male	Female
Ghee	7.18	5.69
Raw G.N. oil ..	6.18	4.72
Refined G.N. oil ..	6.72	5.43
Vanaspati, m. p., 37°C	7.17	5.75
Vanaspati, m.p., 41°C.	7.53	5.49
Basal diet – fat ..	6.01	5.12

There were fewer cases of pathological symptoms in the first generation rats as compared with those in the corresponding animals of Series II. A large number of young ones born in the second generation died or were eaten up within 3 or 4 days of birth. Sufficient number of animals were, however, available for the second generation experiments. No symptoms peculiar to only the vanaspati groups were noticed. Table 9 gives the reproductive ability of female rats of different groups in the first generation.

Table 9: Reproductive and lactating capacities of experimental animals—Series III, first generation

Group	No. of females	No. sterile	No. young born	No. Surviving
Ghee	6	..	41	21
Raw G. N. oil	6	2	22	7
Refined G. N. oil	6	1	33	13
Vanaspati, m. p., 37°C.	6	2	31	14
Vanaspati, m. p., 41°C.	6	..	35	15
Basal diet—fat	6	3	18	3

The results of experiments with the first generation animals show that vitamin supplementation improves the nutritional quality of the poor rice diet, though to a limited extent. Thus the rate of growth and the final weight attained were higher than those of animals of Series II ; there were fewer cases with pathological symptoms ; and there was a definite improvement in reproductive ability.

Second Generation

The 46 surviving young ones of the first generation rats were weaned on the twenty-eighth day. The weights were low (10-13 g.) compared with stock animals of the same age (35-40 g.) and all of them showed symptoms of deficiency — enlarged head, lethargic movement and scanty fur coat. The animals were kept on the experimental diet together with 1 cc. of skimmed milk per rat per day till they attained a weight of about 20 g. They were then fed on the experimental diet. Table 10 gives the average weekly weight increments of male and female rats for 12 weeks.

Table 10: Average weekly increase in weight (in g.) of experimental animals—Series III, second generation

Group	Male	Female
Ghee	6.75	5.00
Raw G. N. oil	5.10	4.00
Refined G. N. oil	5.33	3.30
Vanaspati, m. p., 37°C. ..	5.75	4.41
Vanaspati, m. p., 41°C. ..	5.83	4.16
Basal diet – fat	5.67	4.00

A few pathological symptoms were noticed among animals of the second generation. Two rats (one in the vanaspati, m.p., 41°C. group and one in the basal diet without fat group) died during the experimental period. There were some cases of alopecia in the refined groundnut oil group and one case of acute labyrinthitis in the vanaspati, m.p., 37°C. group. No symptoms peculiar to the vanaspati groups were, however, noticed.

The second generation animals showed poor reproductive and lactating capacities. Most of the female rats conceived late and quite a few were sterile. Of the young ones born, the majority either died or were eaten up by the mothers. Only 8 animals survived for feeding experiments. Table 11 gives the reproductive ability of female rats of the second generation.

Table 11: Reproductive and lactating capacities of experimental animals—Series III, second generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee	6	2	22	..
Raw G. N. oil ..	3	2	2	..
Refined G. N. oil	2	2	..	..
Vanaspati, m. p., 37°C.	4	2	10	4
Vanaspati, m. p., 41°C.	5	3	6	2
Basal diet – fat ..	2	..	9	2

Third Generation

Only 8 animals (4 of the vanaspati, m.p., 37°C. group — 2 males and 2 females ; 2 of the vanaspati, m.p., 41°C. group — 1 male and 1 female ; and 2 of the basal diet without fat group — 1 male and 1 female) were available in the third generation. The animals were very much below weight on the twenty-eighth day when they were weaned. They were fed on experimental diets supplemented with 1 cc. of skimmed milk per rat per day till they attained the proper weight when the milk supplement was stopped and the animals were put on the experimental diet. One of the animals belonging to the vanaspati, m.p., 37°C. group died during the experimental period. Table 12 gives the growth of the rats.

Table 12: Average weekly increase in weight (in g.) of experimental animals—Series III, third generation

Group	Male	Female
Ghee	..	..
Raw G. N. oil	..	..
Refined G. N. oil	..	..
Vanaspati, m. p., 37°C. ..	6.67	5.00
Vanaspati, m. p., 41°C. ..	5.94	5.50
Basal diet – fat ..	7.75	6.33

The growth of rats was poor compared with that of animals of the first generation ; there were no pathological symptoms.

Series IV: Poor Rice Diet Supplemented with Calcium Carbonate

First Generation

Supplementation of the poor rice diet with calcium carbonate slightly improved the nutritional quality in so far as its ability to promote growth was concerned. Table 13 gives the average growth of first generation animals for 12 weeks.

Table 13: Average weekly increase in weight (in g.) of experimental animals—Series IV, first generation

Group	Male	Female
Ghee	6.00	5.21
Raw G.N. oil ..	5.20	4.61
Refined G.N. oil ...	4.90	3.80
Vanaspati, m.p., 37°C. ..	4.25	4.10
Vanaspati, m.p., 41°C. ..	4.75	4.44
Basal diet—fat ..	6.67	6.00

Pathological symptoms similar to those observed in animals fed on poor rice diet containing different fats only (Series II) were observed. There were cases of alopecia in most groups.

The reproductive and lactating capacities were also poor. A large

number of female rats was sterile ; the highest number of sterile animals was found in the 2 vanaspati groups. In the vanaspati, m.p., 37°C. group, none of the animals gave birth to young ones ; in the vanaspati, m.p., 41°C. group, only one female animal conceived. Only 15 young animals were available for the second generation experiments. Table 14 gives the reproductive ability of female rats.

Table 14: Reproductive and lactating capacities of experimental animals — Series IV, first generation

Group	No. of females	No sterile	No. young born	No. surviving
Ghee	6	3	12	7
Raw G.N. oil ..	6	2	14	4
Refined G.N. oil ..	6	2	25	..
Vanaspati, m.p., 37°C. ..	6	6	..	..
Vanaspati, m.p., 41°C. ..	6	5	4	1
Basal diet – fat ..	6	3	16	5

Second Generation

Fifteen animals belonging to ghee, raw groundnut oil, and basal diet without fat groups were available for experiment. No specific pathological symptoms were observed but the growth appeared to be poor compared with that in the first generation animals. Table 15 gives the average growth of rats for 12 weeks.

Table 15: Average weekly increase in weight (in g.) of experimental animals — Series IV, second generation

Group	Male	Female
Ghee	4.66	3.54
Raw G.N. oil ..	5.00	..
Refined G.N. oil ..	..	..
Vanaspati, m.p., 37°C. ..	..	..
Vanaspati, m.p., 41°C. ..	..	..
Basal diet – fat ..	5.83	4.70

After the experimental period, the rats were mated but none of them gave birth to young ones.

SERIES V : POOR RICE DIET SUPPLEMENTED WITH CASEIN

First Generation

The addition of casein improved the nutritional value of the poor rice diet. The growth performance of rats was slightly better than that of animals fed on poor rice diet supplemented with yeast and vitamins. Table 16 gives the average growth for 12 weeks.

Table 16: Average weekly increase in weight (in g.) of experimental animals — Series V: first generation

Group	Male	Female
Ghee	10.00	7.50
Raw G.N. oil ..	8.50	6.50
Refined G.N. oil ..	8.50	6.63
Vanaspati, m.p., 37°C. ..	7.63	6.75
Vanaspati, m.p., 41°C. ..	8.60	6.75
Basal diet – fat ..	10.00	7.20

No pathological symptoms were observed among the experimental animals. All animals were active and the fur coats were smooth.

The reproductive and lactating capacities of the animals were, however, poor. Most of the young ones born either died or were eaten up by the mother rats. Table 17 gives the reproductive ability of first generation animals.

Table 17: Reproductive and lactating capacities of experimental animals — Series V: first generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee	6	1	39	..
Raw G.N. oil ..	6	3	22	3
Refined G.N. oil ..	6	5	10	..
Vanaspati, m.p., 37°C. ..	6	3	14	4
Vanaspati, m.p., 41°C. ..	6	2	21	10
Basal diet – fat ..	6	..	..	..

Second Generation

The young animals in the second generation were very weak. They suffered from scanty fur coat and had poor weight at weaning time (10-14 g.). They were fed on the experimental diet supplemented with 1 cc. of skimmed milk per rat per day till they attained a weight of about 20 g. when the skimmed milk supplement was stopped. They were then fed on the experimental diet. Table 18 gives the average growth of animals for 12 weeks.

Table 18: Average weekly increase in weight (in g.) of experimental animals — Series V: second generation

Group	Male	Female
Ghee	..	..
Raw G.N. oil ..	8.66	5.08
Refined G.N. oil ..	..	..
Vanaspati, m.p., 37°C. ..	7.00	5.00
Vanaspati, m.p., 41°C. ..	6.08	4.42
Basal diet – fat ..	..	..

Series VI: Poor Bengali Diet A

First Generation

The growth was quite satisfactory for 8 weeks. Table 19 shows the results.

Table 19: Average weekly increase in weight (in g.) of experimental animals — Series VI: first generation

Group	Male	Female
Ghee ..	7.50	6.45
Raw G.N. oil ..	5.54	5.35
Refined G.N. oil ..	4.64	4.75
Vanaspati, m.p., 37°C. ..	4.20	5.40
Vanaspati, m.p., 41°C. ..	6.92	6.62
Basal diet – fat ..	7.50	6.90

Slight alopecia was observed in 1 or 2 animals in each group. The rats were active and the fur coats appeared smooth. Distressing symptoms were reported by the Izatnagar workers in the vanaspati group with the poor Bengali diet.

As suggested by the Izatnagar workers the animals were given leafy vegetables and separated milk after the experimental period of 8 weeks. Each rat received daily 5 g. of fresh leafy vegetables and 20 cc. of fresh separated milk as supplements to the diet. The animals were mated after the thirteenth week and the supplements continued till the young ones were 10 days old.

Almost all the rats conceived, though slightly late. Some of the young ones died or were eaten by the mother rats. Sixty two animals were available for the second generation experiments. Table 20 gives the reproductive ability of female rats.

Table 20: Reproductive and lactating capacities of experimental animals — Series VI: first generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee ..	6	..	39	12
Raw G.N. oil ..	6	3	18	10
Refined G.N. oil ..	6	1	34	9
Vanaspati, m.p., 37°C. ..	6	..	38	11
Vanaspati, m.p., 41°C. ..	6	1	33	12
Basal diet – fat ..	6	2	29	8

Second Generation

On the eleventh day after littering, the supplement of fresh vegetables was discontinued and the mother rats were fed with poor Bengali diet A and 20 cc. of separated milk. The young ones were weaned on the twenty-eighth day and put on the experimental diet without any supplement.

The young ones were weak and under-weight (16-18 g.) at weaning time but they picked up weight quickly and were soon in a fairly good condition. Table 21 gives the average weekly growths of the animals for 8 weeks.

Table 21: Average weekly increase in weight (in g.) of experimental animals — Series VI: second generation

Group	Male	Female
Ghee ..	7.37	6.25
Raw G. N. oil ..	6.78	5.75
Refined G.N. oil ..	6.00	4.80
Vanaspati, m.p., 37°C. ..	5.87	5.00
Vanaspati, m.p., 41°C. ..	6.20	5.57
Basal diet – fat ..	7.90	6.87

No pathological symptoms were observed among the second generation animals. On mating all the female animals conceived and a large number of young ones were born. Table 22 gives the reproductive ability of animals of the second generation.

Table 22: Reproductive and lactating capacities of experimental animals — Series VI: second generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee ..	6	..	35	14
Raw G.N. oil ..	5	..	25	17
Refined G.N. oil ..	3	..	18	6
Vanaspati, m.p., 37°C. ..	6	..	40	21
Vanaspati, m.p., 41°C. ..	6	..	25	18
Basal diet – fat ..	4	..	23	17

Third Generation

Many of the young ones born in the third generation died or were eaten up by the mother rats. Sixty one young ones were fed on poor Bengali diet B, which in the uncooked condition, according to the Izatnagar workers, had caused distressing symptoms in vitamin A depleted rats. However, 34 animals were available for studying growth in the third generation. The growth was poor but all the animals survived the experimental period. Table 23 gives the average weekly growths.

Table 23: Average weekly increase in weight (in g.) of experimental animals — Series VI: third generation

Group	Male	Female
Ghee ..	..	3.75
Raw G.N. oil ..	4.25	3.12
Refined G.N. oil ..	..	..
Vanaspati, m p , 37°C. ..	4.00	4.00
Vanaspati, m.p., 41°C. ..	..	3.38
Basal diet – fat ..	4.00	4.16

There were no pathological symptoms among the animals and although the growth was poor the rats were active.

It will be apparent from the results of experiments with poor Bengali diet A that the distressing symptoms, stated to have been observed at Izatnagar, did not develop in these experiments. The Izatnagar workers explained that the distressing symptoms had been obtained not with the poor Bengali diet A, but with a poorer Bengali diet (Poor Bengali diet B) given in the uncooked condition, only fish being fed in the cooked condition. The rats used in those experiments were stated to have been depleted of vitamin A before they were fed on this diet. It was also stated that it was not necessary to continue the experiments to the second and third generations for observing the distressing symptoms, but that the symptoms were noticeable in the first generation itself, between the nineteenth and twentyfifth week. Another series of experiments was, therefore, started using poor Bengali diet B.

Series VII : Poor Bengali Diet B, Cooked

The third generation of young rats obtained from rats fed on poor Bengali diet A were taken to have been depleted of vitamin A, and they were fed on poor Bengali diet B. The diet, however, was given in the cooked condition for the following 2 reasons : (1) rice diets are usually cooked before they are taken by human beings, and (2) participating laboratories other than Izatnagar had also used the cooked diet.

The experiment with cooked poor Bengali diet B was continued for 17 weeks. The growth was poor but all the animals, excepting one fed on basal diet without fat, survived. Table 24 gives the average weekly growths.

Table 24 : Average weekly increase in weight (in g.) of experimental animals — Series VII

Group		Male	Female
Ghee	..	3.40	3.10
Raw G.N. oil	..	3.80	2.80
Refined G.N. oil	..	4.25	3.25
Vanaspati, m.p., 37°C.	..	3.90	3.10
Vanaspati, m.p., 41°C.	..	3.40	3.03
Basal diet-fat	..	4.10	2.90

There were no pathological symptoms ; the fur coats were smooth and the rats were active. No distressing symptoms were obesrved in any animal.

Series VIII : Poor Bengali Diet B, Uncooked

Another series of experiments with uncooked poor Bengali diet B was also carried out. As no depleted young rats were available, young rats from the stock were taken for these experiments. These animals were fed wih uncooked poor Bengali diet B for 11 weeks. Table 25 gives the average weekly growth of the animals. The growth was again poor.

Table 25: Average weekly increase in weight (in g.) of experimental animals — Series VIII

Group	Male	Female
Ghee ..	2.67	2.21
Raw G.N. oil ..	2.35	2.31
Refined G.N. oil ..	2.02	1.89
Vanaspati, m.p., 37°C. ..	2.36	1.61
Vanaspati, m.p., 41°C. ..	2.30	2.13
Basal diet – fat ..	2.21	1.60

No symptoms of a pathological nature were observed.

FAT AND VITAMIN CONTENTS OF LIVERS

After the weaning of the young ones, the parent rats were killed and fat and vitamin A contents of the livers were estimated. Vitamin A was estimated colorimetrically in the Lovibond tintometer.

There was no vitamin A in the livers of animals in any series fed on the poor rice diet. Even the series with yeast and vitamin supplements gave negative results. The percentage of fat in the livers (on dry basis) lay between 6 and 11 and there was no indication of fat infiltration. Positive values for vitamin A were obtained in the livers of animals fed on synthetic diet and on poor Bengali diet A. Tables 26 and 27 give the results of analyses of livers of rats fed on synthetic diet and poor Bengali diet A respectively.

Table 26: Average weight, moisture, fat and vitamin A contents (in Blue Units) of livers of experimental animals fed on synthetic diet

Group	No. of animals	Av. wt. of animals g.	Av. wt. of fresh liver g.	Moisture %	Fat %	Vitamin A Blue Units/g. fresh liver
First generation, males						
Ghee	6	210	7.2	68.1	16.2	0.72
Raw G.N. oil	6	214	6.6	67.5	15.5	0.64
Refined G.N. oil	6	192	6.2	67.8	15.9	0.68
Vanaspati, m.p., 37°C.	5	188	6.3	68.2	16.2	0.70
Vanaspati, m.p., 41°C.	5	186	6.8	67.2	16.7	0.72
Second generation, males						
Ghee	2	168	6.0	67.1	13.5	0.52
Raw G.N. oil	3	153	5.4	65.8	13.2	0.36
Refined G.N. oil	..	..	..	..	..	..
Vanaspati, m.p., 37°C.	2	137	5.2	66·0	12.7	0.46
Vanaspati, m.p., 41°C.	3	142	5.8	66.6	13.1	0.41
First generation, females						
Ghee	6	140	6.2	66.0	15.6	0.68
Raw G.N. oil	6	132	6.0	67.2	15.2	0.73
Refined G.N. oil	6	126	5.8	67.6	16.6	0.72
Vanaspati, m.p., 37°C.	6	127	5.8	66.6	15.8	0.64
Vanaspati, m.p., 41°C.	5	124	5.3	67.3	16.2	0.68
Second generation, females						
Ghee	2	115	5.6	68.1	13.1	0.42
Raw G.N. oil	5	122	6.0	66.4	14.5	0.45
Refined G.N. oil	..	..	..	..	..	..
Vanaspati, m.p., 37°C.	1	111	5.6	67.2	12.7	0.32
Vanaspati, m.p., 41°C.	6	112	5.2	65.6	13.3	0.32

Table 27: Average weight, moisture, fat and vitamin A contents (in Blue Units) of livers of experimental animals fed on poor Bengali diet A

Group	No. of animals	Av. wt. of animals g.	Av. wt. of fresh liver g.	Moisture %	Fat %	Vitamin A Blue Units/g. fresh liver
First generation, males						
Ghee	6	145	6.2	68.0	17.3	1.10
Raw G.N. oil	6	132	5.2	68.4	16.6	0.98
Refined G.N. oil	6	138	5.4	67.2	16.9	1.20
Vanaspati, m.p., 37°C.	6	126	5.3	65.4	17.2	0.84
Vanaspati, m.p., 41°C.	6	136	5.7	66.7	16.8	0.91
Second generation, males						
Ghee	6	147	5.8	64.0	16.2	1.00
Raw G.N. oil	5	148	6.4	65.2	17.3	0.82
Refined G.N. oil	6	136	5.4	67.0	16.5	0.78
Vanaspati, m.p., 37°C.	5	148	6.2	66.5	16.9	0.98
Vanaspati, m.p., 41°C.	6	138	5.1	66.8	17.4	1.10
First generation, females						
Ghee	6	124	5.5	68.8	16.3	1.20
Raw G.N. oil	6	126	5.8	70.2	17.1	0.82
Refined G.N. oil	6	123	5.3	69.1	16.7	1.30
Vanaspati, m.p., 37°C.	6	118	5.6	67.2	16.6	0.94
Vanaspati, m.p., 41°C.	6	123	5.6	68.0	17.2	0.91
Second generation, females						
Ghee	6	118	6.0	67.2	16.9	0.82
Raw G.N. oil	5	112	5.4	64.2	17.0	0.76
Refined G.N. oil	3	108	5.6	65.5	17.2	0.98
Vanaspati, m.p., 37°C.	6	115	4.9	66.3	16.4	0.72
Vanaspati, m.p., 41°C.	6	116	5.7	67.6	16.8	0.78

SECTION II

2. Animal Experiments carried out at the Indian Institute of Science, Bangalore*

The following series of experiments were conducted :

Series I Synthetic diet
Series II Poor rice diet
Series III Poor rice diet + yeast + vitamins A, D, & E
Series IV Poor rice diet + calcium carbonate
Series IV (*a*) Poor rice diet + 1.0 per cent tamarind + 0.1 per cent calcium carbonate
Series IV (*b*) Poor rice diet + calcium lactate and vitamins at sub-optimal levels
Series V Poor rice diet + casein
Series VI Poor Bengali diet A

The observations of growth and reproductive capacity were continued for 3 generations with synthetic diet, for one generation with poor rice diet with or without supplements and for 3 generations with poor Bengali diet A.

In each series there were 60 animals distributed into 5 groups of 12 each, keeping as far as possible equal numbers of sex and littermates in all the groups. The animals were weaned on the twenty-eighth day and kept on the experimental diet (— fat) + 10 cc. of fresh milk for a week. This preliminary feeding with milk was found to be essential in order to enable the young animals to survive for a period of over 12 weeks on the poor diet. Weekly growth records and observations of pathological symptoms were maintained over 12 weeks after which period the males and females of the corresponding groups were paired and allowed to breed in individual cages. When signs of pregnancy were noticed, the females were separated. The time of parturition and lactating capacity were observed. The number of young ones surviving on the twenty-eighth day after parturition was taken as an index of lactating capacity and the total number of young ones born as the index of breeding capacity.

The males were sacrificed on the date of separation from the females after breeding and the livers analysed for vitamin A and fat contents. The females were sacrificed 2 months after parturition and the livers analysed for vitamin A and fat contents.

PREPARATION OF DIETS AND FEEDING

Poor rice diet — As per the composition of the diet given before, the

* The work described in this section was carried out under the supervision of Dr. V. Subrahmanyan, Professor of Biochemistry, Indian Institute of Science, Bangalore, by Drs. C. R. Krishnamurthy and S. M. Bose.

daily requirements of the various ingredients for 60 rats (calculated on the basis of 10 g. of dry diet as optimal daily intake of each rat) were as follows: rice, 471 ; dal, 30 ; common salt, 1.8; non-leafy vegetables, 49.2 ; and leafy vegetables, 12.6 g. The rice was weighed into a clean open vessel and washed twice in running water. Weighed quantities of dal, vegetables and salt were mixed with the rice. Two parts by weight of water for each part of the mixture were added and the vessel was covered with a lid and cooked in boiling water contained in a bigger vessel. Heating for 30 - 40 min. was sufficient for the rice to attain the proper consistency and taste. During the heating, the mixture was frequently stirred to facilitate mixing of ingredients. The cooked food was divided into equal portions by weight and placed in 5 separate open dishes. Six g. of the heated fat were thoroughly mixed with the diet by kneading and the kneaded mass divided into 12 equal parts. When the schedule was strictly adhered to, the proportion of water in the cooked diet did not vary very much from day to day. The supplements given in Series III, IV, IV (*a*) and V were mixed with the cooked diet.

Poor Bengali diet A — All the ingredients excepting fish, egg and meat were cooked in one lot and thoroughly mixed with fish, egg and meat which were separately cooked.

The milk powder was administered separately as a 10 per cent suspension. Yeast was mixed with the cooked diet. Vitamins A, D and E were given orally in propylene glycol solution. The fats under test were heated to 185°C., maintained at that temperature for 5 min., cooled and mixed with the cooked diet.

EXPERIMENTAL RESULTS

Series I : Synthetic diet

First Generation

The growth rates, of animals reared on a synthetic diet are given in Table 1.

TABLE 1 : Average weekly increase in weight (in g.) of experimental animals—Series I, first generation

Group	Male	Female
Ghee	11.81	8.70
Raw G. N. oil	10.38	8.39
Refined G. N. oil	9.29	8.03
Vanaspati, m.p., 37°C.	11.91	7.63
Vanaspati, m.p., 41°C.	10.92	8.76

In contrast to those reared on the poor rice diet, animals of this series behaved normally and compared in general health and activity to stock animals of identical age. De-coating of 1 or 2 animals was observed in the initial state, but the patches were covered up towards the sixth week.

* In this and in the subsequent Tables, only the average figures have been given. The data for individual animals were utilized for statistical analyses.

The results of breeding and lactation are recorded in Table 2.

Table 2: Reproductive and lactating capacities of experimental animals—Series I, first generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee	6	0	45	10
Raw G. N. oil ..	6	0	51	10
Refined G. N. oil ..	6	0	53	11
Vanaspati, m.p., 37°C. ..	6	0	38	10
Vanaspati, m.p., 41°C. ..	6	0	44	10

The gestation period was 28 - 35 days.

Second Generation

The surviving animals from the breeding colony of the first generation of females on synthetic diet were weaned on the twenty-eighth day and put on the experimental diet. In Table 3 are recorded the growth data of the second generation animals.

Table 3: Average weekly increase in weight (in g.) of experimental animals—Series I, second generation

Group	Male	Female
Ghee	11.7	8.1
Raw G. N. oil	11.2	6.7
Refined G. N. oil	9.6	6.6
Vanaspati, m.p., 37°C.	11.8	8.2
Vanaspati, m.p., 41°C.	10.1	7.4

No abnormal symptoms were noticed in any of the groups. The animals were healthy and active throughout.

Table 4 gives the number of young ones born and the number surviving the weaning period.

Table 4: Reproductive and lactating capacities of experimental animals—Series I, second generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee	5	0	32	13
Raw G. N. oil ..	5	1	18	12
Refined G. N. oil ..	8	2	28	16
Vanaspati, m p., 37°C. ..	5	0	31	13
Vanaspati, m.p., 41°C. ..	6	1	29	12

Third Generation

The growth data of the animals of the third generation of rats on synthetic diet are given in Table 5.

Table 5: Average weekly increase in weight (in g.) of experimental animals—Series I, third generation

Group	Male	Female
Ghee	11.5	9.3
Raw G. N. oil	12.1	8.7
Refined G. N. oil	12.4	9.3
Vanaspati, m.p., 37°C.	11.8	9.3
Vanaspati, m.p., 41°C.	11.6	8.6

After the experimental period of 12 weeks the animals were killed and the vitamin A content of the liver was determined.

The males of each generation of the above series were killed after the gestation period and their livers and femur bones excised. Aliquots from the different animals of the same group of the fresh blood-free minced liver were mixed together and used for vitamin A assay. The remaining portions of liver were dried to constant weight at 103°C. and used for fat estimation. For determining vitamin A in the liver, the minced liver was saponified with alcoholic alkali, the unsaponifiables extracted with peroxide-free ether and finally dispersed in chloroform. The blue colour, developed by the addition of a saturated solution of antimony trichloride in chloroform, was measured in a Pulfrich photometer using filter No. S. 61. The vitamin A values are expressed as Blue Units.

The extracted femurs were freed from adhering adipose and tissue matter by mechanical separation and subjected to papain digestion for 24 hours at 50°C., which completely removed the marrow protein matter. The entrained fat was removed by repeated extraction with absolute alcohol followed by ether. The fat-free bones were dried to constant weight at 103°C. and ashed at bright red heat for 4 hours.

The averages of the body weights of animals at the time of dissection, fresh liver weights, weights of femur bones and their ash contents, and fat, moisture and vitamin A values of livers are embodied in Table 6.

Table 6: Results of analysis of livers and femur bones

	No. of animals	Body wt. g.	Wt. of fresh liver g.	Moisture content of liver %	Fat content of liver (on dry basis) %	Vitamin A content Blue units	Wt. of pair of femur bones g.	Ash content of femur bones %
First generation, males								
Ghee	5	184	7.54	67.2	18.2	0.75	0.57	68.6
Raw G. N. oil	6	225	6.82	68.0	18.2	0 81	0.63	68.4
Refined G. N. oil	6	212	6.62	67.0	18.8	0.79	0.61	70.3
Vanaspati m.p., 37°C. ..	6	190	6.24	68.0	18.2	0.70	0.57	68.0
Vanaspati, m.p., 41°C. ..	6	198	7.98	68.3	18.7	0.75	0.60	68.6
Second generation, males								
Ghee	5	221	6.80	67.0	19.1	0.87	0.61	69.8
Raw G. N. oil	5	233	6.80	67.0	18.1	0.84	0.75	68.5
Refined G. N. oil	3	225	6.70	66.0	18.9	0.87	0.70	66.2
Vanaspati, m.p., 37°C. ..	5	238	7.72	68.0	18.4	0.92	0.79	69.2
Vanaspati, m.p., 41°C. ..	4	247	7.05	65.0	18.4	0.87	0.66	67.1
Third generation, males								
Ghee	6	169	7.10	68.3	17.6	0.91	0.57	67.9
Raw G. N. oil	6	168	6.30	67.2	18.2	0.91	0.43	68.2
Refined G. N oil	5	172	7.10	68.4	18.6	0.93	0.47	69.1
Vanaspati, m.p., 37°C. ..	5	169	6.81	68.4	18.9	0.87	0.50	70.2
Vanaspati, m.p., 41°C. ..	6	173	7.20	66.2	17.9	0.92	0.51	69.8
First generation, females								
Ghee	6	154	5.67	70.8	16.4	0.85	0.50	70.4
Raw G. N. oil	6	162	6.41	70.2	18.6	0.87	0.60	69.2
Refined G. N. oil	6	154	5.92	69.8	18.0	1.09	0.50	67.9
Vanaspati, m.p., 37°C. ..	6	153	6 35	69.8	15.2	0.99	0.51	68.8
Vanaspati, m.p., 41°C. ..	4	167	6.47	70.8	16.7	0.89	0.53	69.6
Second generation, females								
Ghee	5	178	6.12	67.2	17.0	0.78	0.51	66.3
Raw G. N. oil	5	203	6.62	71.3	17.9	0.82	0.61	68.0
Refined G. N. oil	8	157	5.89	69.7	18.1	0.81	0 43	68.2
Vanaspati, m.p , 37°C. ..	5	170	5.92	70.1	17.2	0.79	0.48	67.3
Vanaspati, m.p., 41°C. ..	6	144	5.21	64.6	16.9	0.78	0.42	67.4
Third generation, females								
Ghee	6	128	5.19	68.1	17.3	1.02	0.40	63.8
Raw G. N. oil	6	114	4.81	69.8	19.6	0.92	0.39	64.2
Refined G. N. oil	6	114	4.81	69.8	16.9	0.92	0.39	64.2
Vanaspati, m.p., 37°C. ..	6	148	5.20	68.2	16.3	1.10	0.42	64.7
Vanaspati, m.p., 41°C. ..	6	150	5.31	64.6	17.1	1.03	0.44	63.8

Series II : Poor rice diet

First Generation

In Table 7 are given the the average weekly increases in the weights of animals under this series.

Table 7: Average weekly increase in weight (in g.) of experimental animals—Series II, first generation

Group	Male	Female
Ghee	5.10	3.82
Raw G. N. oil	4.92	5.06
Refined G. N. oil ..	5.06	4.12
Vanaspati, m. p., 37°C. ..	5.43	4.67
Vanaspati, m.p , 41°C. ..	4.31	4.33
No fat ..	4.87	3.78

The most characteristic and prominent symptom observed was decoating and this manifested itself in the majority of the animals irrespective of the nature of the fat supplement they received. The animals were far from active and compared poorly with stock animals of the same age. Skin lesions were observed in a few individual animals, but they were covered up when the animals approached maturity. Body twitters and nervous manifestations, characteristic of B-complex deficiency, were also noticed in some animals.

On the completion of the twelfth week of experimentation, the females in each of the 6 groups were allowed to mate with male partners chosen at random. Single pairs of males and females were kept in individual breeding cages. The gestation period ranged from 32 to 56 days. The number of young ones born and their period of survival are recorded in Table 8.

Table 8: Reproductive and lactating capacities of experimental animals—Series II, first generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee	6	2	27	3
Raw G. N. oil	6	3	15	0
Refined G. N. oil	6	3	15	0
Vanaspati, m.p., 37°C. ..	5	1	20	0
Vanaspati, m.p., 41°C. ..	6	3	13	0
No fat	6	5	5	0

Second Generation

Experiments on the second generation could not be carried out as the young ones did not survive the weaning period. Either they died on account of poor lactation or were killed and eaten up by the mothers. The 3 young ones of the ghee group were weighing less than 12 g. each on the twenty-eighth day and died within a week after feeding on the poor rice diet.

The livers of the animals did not give a positive test for vitamin A. The plasma of some animals gave a faint blue colour with antimony trichloride after extraction of the unsaponifiables. The livers did not exhibit any gross microscopic sign of fat infiltration although some of them had a flabby appearance.

Series III : Poor rice diet supplemented with yeast and vitamins

First Generation

The vitamins of the B group were supplied by yeast which was powdered and mixed with the cooked diet. Vitamins A, D and E were given orally in propylene glycol solution. Each rat received per day : thiamine 0.06 mg. ; riboflavin, 0.03 mg. ; nicotinic acid, 0.15 mg.; vitamin A, 60 I. U. ; vitamin D, 10 I. U.; and α-tocopherol acetate, 0.5 mg. The yeast also contained sufficient amounts of pyridoxine and pantothenic acid. In Table 9 are given the growth rates of animals reared for 12 weeks on the vitamin supplemented diet.

Table 9 : Average weekly increase in weight (in g.) of experimental animals—Series III first generation

Group	Male	Female
Ghee	6.45	5.62
Raw G. N. Oil ..	5.58	5.59
Refined G. N. oil ..	6.25	4.54
Vanaspati, m.p., 37°C. ..	5.97	5.47
Vanaspati, m.p., 41°C. ..	5.93	4.79

In general, the animals of this series appeared to be more healthy and active than those receiving the unsupplemented poor rice diet. Most of the deficiency symptoms like de-coating and roughness of the skin were absent. The coat was smooth and glossy except in a few animals which had partial denudation. There was no apparent difference from group to group with regard to the overall condition of the symptoms of skin, tail, paw and eyes of the animals. The supplementation of poor rice diet with vitamins in adequate dosage had helped in a slight improvement of growth and general condition.

The females in each of the groups were allowed to mate at the end of twelve weeks of the experiment. The results are presented in Table 10.

Table 10 : Reproductive and lactating capacities of experimental animals—Series III, first generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee	6	1	32	0
Raw G. N. oil	6	1	28	0
Refined G. N. oil	6	2	16	0
Vanaspati, m. p., 37°C. ..	6	2	17	0
Vanaspati, m. p., 41°C. ..	6	3	16	0

The gestation period in all the groups was over 40 days. The experiment could not be carried through the second generation as the young ones did not survive the weaning period. From Tables 9 and 10 it is evident that the growth rate and reproductive capacity of the animals have improved to a certain extent on supplementation of thir diets with vitamins.

The livers of only certain animals gave a faint blue colour with antimony trichloride indicating that in spite of adequate supplementation of vitamin A, the capacity of the animals for storing the vitamin in the liver had not improved.

SERIES IV: POOR RICE DIET SUPPLEMENTED WITH CALCIUM CARBONATE

First Generation

In Table 11 are given the growth rates of animals receiving the basal rice diet supplemented with calcium carbonate, 0.3 per cent, and the 5 different fats.

Table 11: Average weekly increase in weight (in g.) of experimental animals—Series IV, first generation

Group	Male	Female
Ghee	4.79	4.63
Raw G.N. oil	4.61	4.18
Refined G. N. oil	4.63	4.04
Vanaspati, m.p., 37°C. ..	5.18	4.48
Vanaspati, m.p., 41°C. ..	4.62	3.81

The animals in this series showed symptoms of de-coating and alopecia to the same extent as those reared on the unsupplemented poor rice diet. The coat was rough throughout. Conditions of the skin, eyes, tail and paw were normal. The majority of the animals was dull and sluggish. Of all the deficiencies suffered by animals fed on poor rice diet, the one on which much stress has been laid is that due to calcium. The results of the work of Aykroyd on supplementation of rice diet with calcium lactate have shown that calcium is one of the limiting factors influencing growth. From the results recorded here, it would appear that the nature of the calcium salt is an equally important factor in determining its supplementary value. This aspect has been studied in a separate experiment and the results show that calcium lactate is superior to calcium carbonate in its supplementary value to the rice diet.

The number of young ones born and surviving the weaning period are given in Table 12.

Table 12: Reproductive and lactating capacities of experimental animals — Series IV first generation

Group	No. of females	No. Sterile	No. young born	No. surviving
Ghee	6	2	20	0
Raw G. N. oil	6	2	20	0
Refined G N. oil	6	2	18	0
Vanaspati, m. p., 37°C. ..	7	3	13	0
Vanaspati, m. p., 41°C. ..	5	3	12	0

Here again the gestation period was over 45 days. None of the young ones born survived the weaning time and hence the second generation observations could not be continued.

The Carr-Price reaction of the unsaponifiables of the liver fat was negative in the majority of cases. There was no indication of any gross fat infiltration. The liver weights and fat contents were normal.

Series IV (a): Poor rice diet supplemented with tamarind and calcium carbonate

First Generation

The average weekly increments in the weights of animals under this series are recorded in Table 13. The tamarind was used in the form of a thick soup.

Table 13: Average weekly increase in weight (in g.) of experimental animals — Series IV (a) first generation

Group	Male	Female
Ghee	5.31	4.99
Raw G. N. oil	5.11	5.01
Refined G. N. oil	5.21	4.72
Vanaspati, m.p., 37°C. ..	5.31	4.98
Vanaspati, m.p., 41°C. ..	5.28	4.81

The general health conditions of the animals were not superior to those in Series II. De-coating was prevalent to a slightly smaller extent. It has been independently observed that for securing any noticeable supplementary value, the level of tamarind and chilli should be raised to at least 5 per cent of the diet.

The number of females allowed to mate, the young ones born and surviving are given in Table 14.

Table 14: Reproductive and lactating capacities of experimental animals — Series IV (a) first generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee	6	1	28	3
Raw G. N. oil	6	2	22	2
Refined G. N. oil	6	2	24	0
Vanaspati, m.p., 37°C. ..	6	1	27	3
Vanaspati, m.p., 41°C. ..	6	2	22	0

Series IV (b): Poor rice diet supplemented with calcium lactate and vitamins at sub-optimal levels

Independent of the series of animal experiments described above, it was considered desirable to ascertain whether the poor rice diet could

be improved by supplementation with calcium and vitamins so as to render it suitable for maintaining the animals at a sub-optimal level of health and to enable them to breed during the experimental period. With a view to determining whether the desired sub-optimal condition could be attained by supplementation with calcium and vitamins, the following additions were made to the poor rice diet. Each rat received : (1) 30 mg. of calcium lactate per day, (2) 1 tablet of yeast per week supplying 0.06 mg. nicotinic acid and enough of pyridoxine and pantothenic acid, and (3) 1 mg. of mixed tocopherol per week given as wheat germ oil concentrate. In addition, the proportion of leafy to non-leafy vegetables in the daily diet was made equi-proportional. These supplements cannot be expected to render the deficient diet even half adequate. The average weekly weight increments of animals in this series are given in Table 15.

Table 15 : Average weekly increase in weight (in g.) of experimental animals — Series IV (b) first generation

Group	Male	Female
Ghee	6.44	4.78
Raw G.N. oil	5.77	5.28
Refined G.N. oil	6.49	5.49
Vanaspati, m.p., 37°C. ..	5.97	4.98
Vanaspati, m.p., 41°C. ..	4.82	5.41

The general condition of the animals of this series was definitely better than that of animals under Series II—IV(*a*). The condition of the skin, eyes and paw was normal throughout.

The gestation period was 45—60 days. Table 16 gives the results of breeding.

Table 16 : Reproductive and lactating capacities of experimental animals — Series IV b first generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee	5	1	19	0
Raw G.N. oil	7	2	16	0
Refined G.N. oil	6	2	10	0
Vanaspati, m.p., 37°C. ..	4	1	17	0
Vanaspati, m.p., 41°C. ..	5	1	10	0

Series V : Poor rice diet supplemented with casein

First Generation

The first generation experiments in this series were performed long before Series II, III and IV were completed. The general nutritional level of the stock animals had been improved quite considerably since then. That is apparently the reason why there is no significant difference

between Series II and Series V as is to be expected. The growth rates, of the animals of this series are recorded in Table 17.

TABLE 17: Average weekly increase in weight (in g.) of experimental animals—Series V first generation

Group	Male	Female
Ghee	6.56	5.20
Raw G.N. oil	5.46	5.83
Refined G.N. oil	5.96	5.64
Vanaspati, m.p., 37°C. ..	5.89	4.72
Vanaspati, m.p., 41°C. ..	5.31	4.72

The general condition of the animals was normal throughout. The animals were, however, only slightly superior in appearance and general health to those reared on the poor rice diet alone and compared poorly with stock animals of the same age.

The results of breeding and lactation are given in Table 18.

TABLE 18: Reproductive and lactating capacities of experimental animals—Series V first generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee	7	0	39	0
Raw G.N. oil	6	0	44	3
Refined G.N. oil	4	0	24	0
Vanaspati, m.p., 37°C. ..	6	0	41	3
Vanaspati, m.p., 41°C. ..	6	0	42	3

The gestation period was 28 to 42 days. Only a few animals survived the weaning period showing thereby that even after supplementation with protein, the rice diet does not ensure efficient lactation.

As in the previous series, the majority of the livers of the animals gave negative Carr-Price reaction. The appearance of the livers otherwise was normal.

Series VI: Poor Bengali Diet A

First Generation

The animals were placed on the diet for the first 8 weeks of experimentation. Subsequently, each rat received a daily supplement of 20 cc. of fresh skimmed milk and 1 g. of air-dry lucerne powder (corresponding to 5 g. of fresh lucerne) till the animals were mated and the young ones born were 10 days old. The mating was done after the animals had attained 4 months of age. From the eleventh day after birth of litters, the green was discontinued and the rats were fed on the usual diets + 20 cc. of skimmed milk. The litters were weaned on the twenty-eighth day and straightaway put on the experimental diet without the supplements.

The growth rates and related data and results of breeding over 3 generations are given in Tables 19—23.

TABLE 19: Average weekly increase in weight (in g.) of experimental animals—Series VI, first generation

Group	Male	Female
Ghee	11.93	9.83
Raw G.N. oil	11.12	9.80
Refined G.N. oil	10.80	8.98
Vanaspati, m.p., 37°C.	11.40	9.91
Vanaspati, m.p., 41°C.	11.86	9.48

TABLE 20: Reproductive and lactating capacities of experimental animals—Series VI, first generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee	6	0	46	27
Raw G.N. oil	6	0	45	32
Refined G.N. oil	6	0	52	27
Vanaspati, m.p., 37°C.	6	0	49	34
Vanaspati, m.p., 41°C.	6	0	58	29

TABLE 21: Average weekly increase in weight (in g.) of experimental animals—Series VI, second generation

Group	Male	Female
Ghee	9.6	6.6
Raw G.N. oil	9.5	6.5
Refined G N. oil	8.5	6.4
Vanaspati, m p., 37°C.	7.5	6.5
Vanaspati, m.p., 41°C.	8.1	7.0

TABLE 22: Reproductive and lactating capacities of experimental animals—Series VI, second generation

Group	No. of females	No. sterile	No. young born	No. surviving
Ghee	6	0	30	20
Raw G.N. oil	6	1	22	13
Refined G.N. oil	5	0	29	18
Vanaspati, m.p., 37°C.	5	0	29	15
Vanaspati, m.p., 41°C.	6	1	33	19

TABLE 23: Average weekly increase in weight (in g.) of experimental animals—Series VI, third generation

Group	Male	Female
Ghee	8.6	7.0
Raw G.N. oil	8.6	6.6
Refined G.N. oil	8.7	6.3
Vanaspati, m.p., 37°C.	9.3	6.6
Vanaspati, m.p., 41°C.	8.9	6.0

During the first 3 to 4 weeks the animals were rather dull and sluggish although they were apparently superior to those thriving on the poor rice diet. De-coating was observed in many animals. From the ninth week onwards there was a remarkable improvement. The animals became more active and approached the standard of stock animals. No untoward symptoms were observed in second or third generation animals.

The results of liver assay of fat and vitamins and bone ash determinations are embodied in Table 24.

TABLE 24: Results of analysis of liver and femur bones—Poor Bengali diet A

Group	No. of animals	Body wt.	Wt. of fresh liver	Moisture content	Fat content (dry basis)	Vitamin A Blue Units	Wt. of pair of femur bones	Ash content of bones
		g.	g.	%	%		g.	%
First generation, males								
Ghee	6	165	7.34	71.2	18.0	1.92	0.56	70.7
Raw G.N. oil	5	159	6.19	70.2	18.0	1.58	0.49	69.6
Refined G.N. oil	6	147	5.29	68.7	18.2	1.08	0.54	70.1
Vanaspati, m.p., 37°C.	5	146	5.79					
Vanaspati, m.p., 41°C.	6	160	5.47					
Second generation, males								
Ghee	6	174	5.80	69.7	18.7	1.83	0.43	79.3
Raw G.N. oil	6	203	7.12	71.4	18.7	1.62	0.48	71.0
Refined G.N. oil	6	187	6.02	70.2	19.2	1.37	0.45	72.0
Vanaspati, m.p., 37°C.	6	189	6.12	71.3	19.7	1.69	0.45	71.0
Vanaspati, m.p., 41°C.	6	174	5.89	69.8	19.9	1.62	0.43	69.8
Third generation, males								
Ghee	6	134	5.39	70.8	18.1	1.35	0.41	70.2
Raw G.N. oil	6	133	5.64	71.3	18.8	1.43	0.40	72.0
Refined G.N. oil	5	128	5.37	70.8	18.6	1.32	0.39	68.7
Vanaspati, m.p., 37°C.	6	139	5.90	70.9	18.9	1.30	0.39	68.7
Vanaspati, m.p., 41°C.	5	130	5.40	71.4	18.9	1.31	0.41	69.4
First generation, females								
Ghee	3	140	5.24	70.6	18.0	1.48	0.43	72.6
Raw G.N. oil	5	148	5.52	72.4	17.4	1.79	0.40	74.3
Refined G.N. oil	6	129	4.33	70.6	17.0	0.99	0.37	73.0
Vanaspati, m.p., 37°C.	6	133	6.99	69.7	18.1	0.87	0.42	70.2
Vanaspati, m.p., 41°C.	6	150	5.78	70.3	18.3	0.87	0.41	67.7
Second generation, females								
Ghee	6	148	4.87	71.4	17.6	1.63	0.42	70.9
Raw G.N. oil	6	132	4.82	72.9	17.6	1.63	0.41	74.6
Refined G.N. oil	5	155	5.20	71.6	16.8	1.71	0.43	73.2
Vanaspati, m.p., 37°C.	6	147	4.90	70.8	17.9	1.67	0.42	71.6
Vanaspati, m.p., 41°C.	6	150	4·98	70.4	18.2	1.59	0.43	72.8
Third generation, females								
Ghee	6	109	4.21	70.4	17.9	1.17	0.38	70.9
Raw G.N. oil	6	109	4.12	71.8	18.1	1.12	0.37	73.8
Refined G.N. oil	7	101	4.03	70.6	18.0	1.08	0.36	72.7
Vanaspati, m.p., 37°C.	6	103	4.07	69.8	18.9	1.21	0.35	71.8
Vanaspati, m.p., 41°C.	5	94	3.98	69.7	18.7	1.10	0.32	71.7

Comparative influence of animal and vegetable fats on the biological value of dietary protein and absorption of minerals (adult rats)

In view of the conflicting opinions on the role of fat in the utilisation of dietary protein and minerals, it was of interest to determine the exact function of fat in the assimilation of nutrients by the usual metabolism studies under controlled conditions. Six adult rats were put through metabolism trials on the following diet: corn starch, 50 ; cane sugar, 15; fat—, vitamins—, and ash-free casein, 15 ; Osborne and Mendel salt mixture, 5 ; yeast, 5 ; and fat to be tested, 10 per cent.

For the protein—, or fat-free diet, an equal amount of corn starch replaced the protein or fat. In addition, each rat received a daily dose of 60 I. U. of vitamin A, 10 I. U of vitamin D and 0.5 mg. α-tocopherol acetate dissolved in propylene glycol.

The rats were about 4 months old and the weights of the 3 females were about 150 g., and those of the 3 males nearly 200 g. Each metabolism study lasted for 7 days, the first 4 days being considered as the initial acclimatisation period and the last 3 as the regular collection period. Between the metabolism studies, the animals were given a rest period of 1 week when they received the usual stock diet. The experimental procedure for collecting faeces and urine and the analytical methods were the same as those followed in these laboratories.

Tables 25 and 26 summarise the data on nitrogen, calcium and phosphorus balances of the animals.

Table 25 : Metabolism studies

1. Endogenous nitrogen excretion on protein-free diet supplemented with 10 per cent fat*

Rat no.	Wt. g.	Daily food intake g.	Urine N mg.	Faecal N mg.
1F	144	9.45	36.1	20.9
2F	151	9.05	37.9	21.3
3F	148	8.41	36.1	11.7
4M	225	8.71	39.9	21.7
5M	211	9.51	37.6	10.3
6M	220	9.35	39.9	14.6

* An independent experiment on endogenous nitrogen excretion under the influence of different fats at 5 per cent level revealed no significant differences; hence, in the present study, only ghee was used as the source of fat.

2. Nitrogen excretion on 15 per cent casein diet supplemented with 10 per cent ghee

Rat no.	Daily food intake g.	Intake of N mg.	Urine N mg.	Faecal N mg.	Digest. coefft. %	Biological value %
1	5.58	123.4	64.7	17.9	100.0	77.1
2	6.84	151.4	75.9	25.7	97.1	74.2
3	5.01	110.9	57.4	19.9	93.3	78.6
4	8.64	191.3	79.7	35.8	92.6	77.5
5	7.58	168.0	79.3	27.2	90.1	72.7
6	8.81	195.0	84.9	42.2	85.8	73.6
					93.2	75.6

3. Nitrogen excretion on 15 per cent casein diet supplemented with 10 per cent raw G.N. oil

Rat no.	Daily food intake g.	Intake of N mg.	Urine N mg.	Faecal N mg.	Digest. coefft. %	Biological value %
1	8.68	192.1	72.6	37.5	91.4	79.2
2	9.68	214.2	77.8	29.9	96.0	80.6
3	9.88	218.7	82.6	38.0	88.0	75.8
4	9.35	206.9	79.7	39.3	91.5	79.0
5	11.08	245.1	83.5	50.2	83.7	75.8
6	10.51	232.7	87.3	36.9	89.6	77.3
					90.0	78.0

4. Nitrogen excretion on 15 per cent casein diet supplemented with vanaspati, m.p., 37°C.

1	10.34	226.5	93.0	34.0	94.2	73.3
2	8.54	188.9	81.6	25.1	98.0	76.4
3	10.40	230.0	74.0	37.5	88.5	81.5
4	9.54	211.1	86.3	42.1	88.3	77.5
5	8.87	196.3	81.4	25.2	92.4	75.9
6	10.27	227.3	90.8	35.9	90.6	75.3
					92.0	76.6

5. Nitrogen excretion on 15 per cent casein diet supplemented with coconut oil, 10 per cent

1	6.28	139.1	65.0	21.6	99.6	79.1
2	6.35	140.5	63.4	32.7	91.9	80.2
3	5.78	128.0	56.8	21.7	92.9	82.5
4	8.32	184.1	85.3	28.7	96.2	74.3
5	7.85	140.4	62.3	34.0	83.1	78.8
6	7.45	164.9	79.9	27.9	91.9	73.6
					92.6	78.1

6. Nitrogen excretion on fat-free diet containing 15 per cent casein

1	6.3	148.7	91.1	20.9	87.7	60.8
2	6.4	154.5	79.7	32.5	83.2	66.2
3	5.8	153.1	77.3	23.9	90.4	62.3
4	8.3	176.7	106.3	15.2	98.2	58.5
5	7.9	215.0	103.4	45.4	84.2	59.7
6	7.5	157.5	80.8	21.8	92.6	61.5
					89.4	61.5

7. Nitrogen excretion on protein-free and fat-free diet

Rat no.	Daily food intake g.	Urine N mg.	Faecal N mg.
1	11.84	39.9	2.7
2	8.86	36.2	6.5
3	11.93	22.8	11.5
4	12.02	34.1	12.5
5	10.82	30.4	11.3
6	9.77	24.7	10.2

Table 26: Phosphorus and calcium excretion

1. Protein-free diet supplemented with 10 per cent fat

Rat no.	Phosphorus			Calcium		
	Daily intake mg.	Urine P mg.	Faecal P mg.	Daily intake mg.	Urine Ca mg.	Faecal Ca mg.
1	38.4	10.4	24.6	55.7	2.5	52.8
2	36.8	9.2	34.3	53.4	2.3	51.9
3	34.2	10.3	23.8	49.6	2.4	44.0
4	35.4	10.1	19.4	51.5	3,1	46.8
5	38.7	14.3	27.5	56.1	1.9	52.8
6	38.0	13.6	26.9	55.1	2.8	50.9

2. 15 per cent casein diet supplemented with 10 per cent ghee

1	28.3	5.9	18.9	35.0	4.3	29.5
2	34.7	3.8	22.9	42.8	1.6	40.1
3	20.2	3.6	20.0	31.4	4.1	26.8
4	43.9	8.3	30.2	54.3	3.6	50.0
5	48.4	10.6	24.9	47.6	3.9	43.1
6	45.0	9.8	27.6	55.3	3.8	51.2

3. 15 per cent casein diet supplemented with 10 per cent G.N. oil

1	45.6	3.0	27.3	54.5	3.5	50.2
2	50.8	1.9	18.1	60.8	2.9	51.7
3	51.9	3.0	26.9	62.0	3.2	50.2
4	49.0	3.6	28.1	58.7	3.2	52.0
5	58.2	5.3	32.6	69.3	2.6	62.0
6	53.2	4.5	27.4	65.9	3.3	53.3

4. 15 per cent casein diet supplemented with 10 per cent vanaspati, m.p., 37°C.

1	58.7	6.1	24.3	64.2	4.5	51.5
2	49.0	5.4	21.3	53.6	4.0	43.0
3	59.7	5.0	28.1	65.3	3.8	58.0
4	54.7	10.1	31.3	59.9	3.8	52.0
5	50.9	8.8	21.5	56.0	3.3	48.4
6	58.9	9.5	28.8	64.1	3.6	52.3

5. 15 per cent casein diet supplemented with 10 per cent coconut oil

1	33.2	4.8	21.6	39.3	3.4	33.1
2	33.8	4.8	14.4	39.8	3.3	31.6
3	25.1	3.9	19.0	36.3	3.1	30.1
4	42.2	8.0	19.5	52.3	3.3	39.2
5	49.7	9.2	16.9	49.3	2.6	41.3
6	40.9	8.3	20.4	46.8	2.8	37.0

6. Protein-free and fat-free diet

1	35.3	4.9	29.3	62.7	3.6	59.4
2	36.4	5.4	20.1	46.9	3.3	47.7
3	35.6	3.4	29.0	63.4	3.1	64.2
4	35.9	7.9	25.1	63.6	3.3	66.0
5	32.3	7.4	26.5	57.3	3.2	61.0
6	29.2	6.3	21.1	51.4	3.9	54.4

7. 15 per cent casein diet free of fats

1	35.2	6.2	27.4	42.2	0.6	40.9
2	36.6	6.9	31.5	44.1	0.5	43.2
3	36.3	6.4	18.7	43.4	1.7	41.6
4	40.9	8.7	25.9	50.1	0.5	50.6
5	50.9	14.2	23.0	60.9	1.7	59.6
6	37.3	8.8	23.6	44.6	1.7	42.8

Comparative influence of butterfat and vanaspati, m.p., 37°C, on calcium and phosphorus absorption in the growing rat

Twelve young rats from the stock colony were weaned on the twenty-eighth day and maintained on the stock diet — 10 cc. milk for a week after which period they were divided into 2 groups of 6 each (3 males and 3 females). The animals were then put on the following diet: Corn starch, 50 ; extracted casein, 15 ; Osborne Mendel salt mixture, 5 ; yeast, 5 ; cane sugar, 15 ; and fat to be tested 10 per cent. Each rat received a daily supplement of 60 I. U. of vitamin A, 10 I. U. of vitamin D and 0·5 mg. ∝-tocopherol acetate in 0.2 cc. of propylene glycol.

The growth of the animals was observed during a period of 12 weeks. The food consumed during the period was noted. The excretion of calcium and phosphorus in urine and faeces was estimated at 3 different stages during the period.

TABLE 27: Rate of growth of animals on synthetic diet + 10 % fat over 12 weeks

No. and Sex	Initial wt.	Final wt.	Food consumed	Growth/g. of protein	Av. weekly increase in wt.
	g.	g.	g.	g.	g.
Butterfat group					
1 Female	40	150	655	1.12	9.2
2 do.	40	138	662	1.98	8.2
3 do.	45	151	672	1.05	8.8
4 Male	33	170	662	1.36	11.4
5 do.	47	180	663	1.32	11.1
6 do.	31	152	651	1.23	10.0
				1.01	9.8
Vanaspati group					
1 Female	42	131	658	0.92	7.4
2 do.	36	115	655	0.81	6.6
3 do.	40	150	655	1.12	9.2
4 Male	35	154	655	1.21	10.0
5 do.	32	170	660	1.39	11.5
6 do.	41	185	662	1.45	12.0
				1.15	9.5

Table 28: Food intake and faecal excretions (for 10-day periods) during 3 stages of the metabolism study

No. and sex	Butterfat group: Food consumed g.	Butterfat group: Faecal excretion g.	No. and sex	Vanaspati group: Food consumed g.	Vanaspati group: Faecal excretion g.
Stage I: 3 weeks					
1 Female	24.3	1.85	7 Female ..	22.6	1.53
2 do. ..	21.7	1.76	8 do. ..	21.4	1.38
3 do. ..	23.4	1.88	9 do. ..	21.4	2.61
4 Male ..	22.8	1.92	10 Male ..	22.5	1.39
5 do. ..	21.5	2.19	11 do. ..	22.1	2.12
6 do. ..	22.9	1.09	12 do. ..	21.2	2.29
Stage II: 6 weeks					
1 Female	35.0	2.56	7 Female ..	34.0	2.95
2 do. ..	34.0	2.46	8 do. ..	34.0	2.85
3 do. ..	34.5	2.62	9 do. ..	34.5	3.15
4 Male ..	35.5	2.94	10 Male ..	35.0	2.00
5 do. ..	36.0	2.89	11 do. ..	35.0	2.77
6 do. ..	35.0	2.29	12 do. ..	34.5	3.96
Stage III: 9 weeks					
1 Female	35.5	2.41	7 Female ..	35.5	2.93
2 do. ..	35.0	2.20	8 do. ..	36.4	1.95
3 do. ..	36.5	2.25	9 do. ..	35.2	2.64
4 Male ..	37.0	3.08	10 Male ..	35.5	2.76
5 do. ..	36.5	2.53	11 do. ..	35.0	3.41
6 do. ..	35.5	2.40	12 do. ..	33.5	3.86

Table 29: Calcium and phosphorus balances for 4-day periods during 3 stages of metabolism

No. and sex	Calcium excretion: Intake mg.	Urine mg.	Faecal mg.	Absorption %	Phosphorus excretion: Intake mg.	Urine mg.	Faecal mg.	Absorption %
Stage I: 3 weeks								
Butterfat group								
1 Female	247.9	7.4	136.1	45.1	137.3	15.7	61.5	55.2
2 do.	221.3	9.1	142.0	35.8	123.7	20.0	71.5	42.2
3 do.	238.7	6.9	133.8	44.0	133.4	19.1	66.5	50.0
4 Male	232.6	9.1	143.1	38.5	130.0	22.5	63.6	50.0
5 do.	219.3	9.8	127.8	41.7	122.6	25.7	84.3	31.2
6 do.	233.6	6.3	121.2	48.2	130.5	16.6	63.6	49.0
Vanaspati group								
7 Female	230.5	3.6	113.2	50.9	128.8	18.4	48.6	62.3
8 do.	217.3	3.3	96.8	55.7	122.0	12.3	51.4	57.9
9 do.	218.3	4.3	198.1	55.2	122.0	17.3	94.2	22.8
10 Male ..	229.5	2.7	114.7	50.3	128.3	9.9	51.3	60.0
11 do.	229.5	4.2	136.9	40.3	126.0	25.4	48.3	60.0
12 do.	216.4	6.6	123.1	43.1	120.8	21.1	76.5	36.5

Stage II : 6 weeks

Butterfat group									
1	Female	347.0	3.7	114.4	68.0	198.5	19.3	87.2	56.1
2	do.	346.8	3.6	112.9	67.5	193.8	17.7	96.4	50.3
3	do.	352.9	2.3	128.4	63.6	196.7	16.8	109.3	44.4
4	Male	362.1	2.1	128.6	64.6	202.4	12.5	102.9	49.8
5	do.	367.2	3.8	109.8	70.1	205.2	24.8	102.9	50.0
6	do.	357.0	2.8	119.3	66.6	198.5	11.1	85.7	56.8
Vanaspati group									
7	Female	347.8	5.3	123.4	64.5	193.8	15.9	97.2	49.8
8	do.	347.8	6.2	122.4	64.8	193.8	16.1	94.2	51.4
9	do.	351.9	3.1	121.2	65.6	196.7	23.8	102.1	48.1
10	Male	357.0	2.4	93.8	73.7	198.5	14.3	77.2	61.1
11	do.	357.0	5.6	126.1	64.6	198.5	40.6	102.8	48.2
12	do.	351.9	3.1	124.4	64.6	196.7	31.5	102.8	47.1

State III : 9 weeks

Butterfat group									
1	Female	362.1	3.0	120.1	66.8	202.4	21.1	101.5	49.8
2	do.	357.0	3.4	115.6	67.6	198.5	23.9	85.7	56.8
3	do.	372.3	6.1	106.3	71.4	208.3	27.3	86.5	57.0
4	Male	377.4	3.1	134.8	64.3	210.9	30.2	103.6	50.0
5	do.	372.3	3.1	116.6	68.7	208.3	27.3	82.2	61.0
6	do.	362.1	3.5	117.8	67.5	202.4	26.4	82.2	60.2
Vanaspati group									
7	Female	362.1	2.8	102.2	66.7	202.4	16.6	95.0	53.1
8	do.	371.4	2.9	94.7	74.5	208.1	20.2	72.8	65.0
9	do.	359.0	3.7	98.1	72.7	201.3	18.8	99.3	51.8
10	Male	362.1	3.4	107.8	70.1	202.4	21.8	93.6	52.9
11	do.	357.0	3.6	125.8	64.8	198.5	25.2	97.8	51.0
12	do.	341.7	2.8	129.8	62.0	201.0	31.8	100.0	50.0

Table 30: Results of analysis of livers and femur bones

No. and sex		Wt. of animal	Wt. of fresh liver	Moisture content	Fat content (dry basis)	Vitamin A Blue units	Wt. of pair of femur	Ash in femur
		g.	g.	%	%		g.	%
Butterfat group								
1	Female	156	5.67	69.3	16.2	1.12	0.5213	71.3
2	do.	140	7.26	69.8	16.8	1.13	0.5106	71.8
3	do.	155	6.26	69.7	16.9	1.12	0.5189	70.3
4	Male	180	6.38	69.2	15.4	1.08	0.5462	69.8
5	do.	185	9.48	69.8	15.9	1.09	0.5473	69.1
6	do.	165	7.90	69.3	16.3	1.10	0.5191	69.9
Vanaspati group								
7	Female	137	4.93	68.2	15.1	1.10	0.5032	71.4
8	do.	118	5.62	68.7	16.2	1.12	0.4937	70.9
9	do.	150	6.53	68.3	15.8	1.07	0.5316	70.3
10	Male	158	4.85	69.1	15.9	1.06	0.5343	70.4
11	do.	172	7.12	69.2	15.3	1.07	0.5628	69.3
12	do.	190	7.90	68.7	15.6	1.10	0.5987	70.2

SECTION III

Animal Experiments carried out at the Indian Veterinary Research Institute, Izatnagar*

The following series of experiments were conducted :

Series	I	Synthetic diet
Series	II	Poor rice diet
Series	III	Poor rice diet + yeast and vitamins A, D and E
Series	IV	Poor rice diet + calcium carbonate
Series	V	Poor rice diet + casein
Series	V(a)	Poor rice diet + yeast + vitamins + calcium carbonate + casein
Series	VI	Poor Bengali diet B (uncooked)

The experimental animals were weaned from the stock colony at 28 days of age and were divided into groups of 12 animals — 6 males and 6 females. For each diet there were 5 groups, each group getting one of the 5 test oils. Littermates were distributed as evenly as possible between the different groups, and comparable groups were so constituted as to have nearly equal average initial weights. All groups of the same diet were as far as possible started together to eliminate the effect of weather and other variables.

PREPARATION OF THE DIET AND FEEDING

The oils and fats were heated for 5 minutes at 180°—200°C., cooled and stored for a week.

Synthetic Diet

The day's requirement of the individual animals was weighed out in a porcelain cup and mixed with the requisite quantity of fat. Water was added to bring the mass to a creamy consistency and fed to the animals.

Poor rice diet (supplemented and unsupplemented)

Rice and dal were separately crushed in a milling machine. The vegetables were chopped and passed through a mincer. The day's diet was cooked for 40 minutes in a covered vessel to make a soft mass. Yeast was added to the cooked diet while still warm. The requisite quantity of fat was added and the mass made homogeneous and fed to the animals.

Bengali diet B (uncooked)

Only fish was boiled, dressed and bones removed. Rice and dal were separately crushed in a milling machine and the vegetables chopped and passed through a mincer. The day's require-

* The work described in this section was carried out under the supervision of Dr. N.D. Kehar, Officer-in-Charge, Animal Nutrition Section, Indian Veterinary Research Institute, Izatnagar. The experimental work was carried out by Shri K. Sahai.

ment of all the components of the diet, in proper proportion, was taken and thoroughly mixed. To 95 parts of the mixture was added 5 parts of the heated and cooled test oil or fat. This was fed to individual animals after making a paste with the required amount of water.

Vitamins A, D and E were separately made up to a known volume with propylene glycol and a volume containing the requisite quantity was fed to the animals orally once a week.

The synthetic and the poor rice diet experiments were carried out over 3 generations and the Bengali diet experiment over one generation only.

The experimental procedure in the case of synthetic and poor rice diets supplemented and unsupplemented was, as approved by the Committee, started one week after weaning, whereas the Bengali diet rats were put on the experimental diet immediately after weaning. During the one week of pre-experimental period, the rats of the synthetic diet series were fed exactly the same diet as during the experimental feeding period. The animals of the poor rice diet series, supplemented and unsupplemented, during the one week of pre-experimental period, received in addition to the usual experimental diet, 10 cc. of 10 per cent. suspension of Klim whole milk powder per head per day in the first generation and 5 cc. in the second and third generations. During the experimental feeding period, all the 3 generations of animals of the poor rice diet series, supplemented and unsupplemented, were given 1 cc. of 10 per cent suspension of Klim whole milk powder per head per day, in addition to the experimental diet.

The litters obtained from the rats maintained on a vitamin A-low diet were weaned at 4 weeks and put on the poor Bengali diet B; feeding on the experimental diet was continued for 23 weeks.

The experimental animals were fed *ad libitum* and the intake was measured thrice every fortnight. The animals were weighed individually on the day of commencement of the experiment and twice every week thereafter. All the rats were examined every few days for pathological symptoms. Experimental feeding was continued for a period of 12 weeks except in the case of the Bengali diet B in which the study was continued for 23 weeks.

RESULTS AND GENERAL REMARKS

Growth of rats

The data relating to the average daily food consumption are presented in Table 1. The average weekly increases in the weights of male and female rats of the different fat groups, Series I to V(a), for the three generations are recorded in Tables 2 to 7; Table 8 gives data relating to the first generation in Series VI.

***TABLE 1: Average food consumption per rat per day during the 12 weeks of experimental feeding**

	Series I: Synthetic diet		Series II: Poor rice diet		Series III: Poor rice diet+yeast		Series IV: Poor rice diet+calcium carbonate		Series V: Poor rice diet+casein		Series V(a): Poor rice diet+yeast+calcium carbonate+casein	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
First Generation												
Ghee	9.5	7.0	6.3	5.8	11.5	9.1	7.0	6.7	5.5	5.5	9.9	7.1
Raw G. N. oil	9.0	7.4	5.0	5.3	10.8	8.4	5.6	6.1	5.4	5.8	10.0	7.5
Refined G. N. oil ..	9.6	7.6	5.6	4.7	10.1	8.0	6.3	5.7	4.8	5.2	10.4	8.2
Vanaspati, m.p., 37°C. ..	9.8	7.1	4.6	5.4	12.5	8.6	5.6	5.6	4.6	5.3	11.7	7.8
Vanaspati, m. p. 41°C. ..	10.2	7.5	5.3	5.6	11.4	8.3	5.0	6.3	5.5	6.2	11.1	7.9
Second Generation												
Ghee	5.5	6.2	5.7	5.2	10.3	9.7	6.0	7.0	5.9	7.1	10.6	9.2
Raw G. N. oil	4.4	4.4	5.9	5.5	9.4	8.9	5.5	7.1	6.6	6.2	11.7	8.9
Refined G. N. oil ..	2.9	3.8	4.6	5.3	8.8	8.9	6.3	7.0	6.3	6.7	10.9	9.3
Vanaspati, m. p., 37°C. ..	5.5	4.2	5.4	5.7	10.2	9.6	6.9	6.6	6.0	6.3	11.2	11.2
Vanaspati, m. p., 41°C. ..	4.6	5.0	4.8	5.0	9.3	9.2	6.4	7.4	5.8	6.9	12.1	10.3
Third Generation												
Ghee	7.5	7.8	6.7	6.3	11.5	10.1	8.1	8.8	7.1	7.1	11.4	10.1
Raw G. N. oil	3.6	3.0	6.0	6.3	10.9	9.8	7.4	7.7	7.1	7.1	11.3	10.2
Refined G. N. oil ..	..	..	5.5	5.6	11.0	9.8	7.6	7.6	7.1	7.0	10.9	10.5
Vanaspati, m. p., 37°C. ..	..	..	5.6	6.0	11.5	10.8	7.5	8.4	6.9	7.1	11.0	10.5
Vanaspati, m. p., 41°C. ..	..	..	5.7	5.9	11.0	10.1	7.7	8.3	7.6	7.7	10.8	10.0

* In this and the subsequent Tables, only the average figures have been given. The data for individual animals have been utilised for statistical analyses.

Table 2: Average weekly increase in weight of experimental animals—Series I

Group	First generation		Second generation		Third generation	
	Male	Female	Male	Female	Male	Female
	g.	g.	g.	g.	g.	g.
Ghee	14.69	7.93	8.35	7.37	8.14	7.75
Raw G. N. oil ..	13.55	8.33	3.59	3.33	3.93	3.39
Refined G. N. oil ..	13.42	8.47	1.76	..	..	..
Vanaspati, m. p., 37°C.	13.13	8.05	..	..	..	..
Vanaspati, m. p., 41°C.	13.90	8.25	..	3.21	..	..

Table 3: Average weekly increase in weight of experimental animals—Series II

Group	First generation		Second generation		Third generation	
	Male	Female	Male	Female	Male	Female
	g.	g.	g.	g.	g.	g.
Ghee	4.53	4.26	5.16	4.29	4.44	4.36
Raw G. N. oil ..	3.75	3.01	4.10	4.18	3.71	3.90
Refined G. N. oil ..	3.11	2.70	3.83	4.36	3.23	3.29
Vanaspati, m. p., 37°C.	2.98	3.58	4.00	4.68	3.38	3.88
Vanaspati, m. p., 41°C.	3.10	3.84	3.41	3.36	3.75	4.31

Table 4: Average weekly increase in weight of experimental animals—Series III

Group	First generation		Second generation		Third generation	
	Male	Female	Male	Female	Male	Female
	g.	g.	g.	g.	g.	g.
Ghee	14.76	9.62	9.75	7.82	12.32	9.15
Raw G. N. oil ..	12.16	8.28	9.80	7.01	11.73	7.94
Refined G. N. oil ..	10.95	8.09	8.77	6.74	11.46	6.96
Vanaspati, m. p., 37°C.	12.41	9.00	9.31	7.34	13.18	9.03
Vanaspati, m. p., 41°C.	12.49	8.00	8.38	7.07	11.23	7.84

Table 5: Average weekly increase in weight of experimental animals—Series IV

Group	First generation		Second generation		Third generation	
	Male	Female	Male	Female	Male	Female
	g.	g.	g.	g.	g.	g.
Ghee	6.71	5.43	5.28	5.26	6.33	6.03
Raw G. N. oil ..	4.04	4.68	5.21	5.68	4.55	5.27
Refined G. N. oil ..	4.08	4.58	4.65	5.17	5.55	5.32
Vanaspati, m.p., 37°C.	2.97	3.97	3.99	4.47	4.90	5.77
Vanaspati, m.p., 41°C.	3.39	4.77	3.72	5.27	4.91	5.39

Table 6: Average weekly increase in weight of experimental animals—Series V

Group	First generation		Second generation		Third generation	
	Male	Female	Male	Female	Male	Female
	g.	g.	g.	g.	g.	g.
Ghee	3.88	5.05	5.69	5.32	6.72	5.92
Raw G. N. oil ..	3.20	4.58	6.11	5.74	5.74	5.03
Refined G. N. oil ..	3.33	4.55	5.83	5.55	6.13	5.03
Vanaspati, m.p., 37°C.	3.69	3.68	5.71	5.06	6.37	5.53
Vanaspati, m.p., 41°C.	3.98	4.74	4.53	5.21	6.84	5.67

Table 7: Average weekly increase in weight of experimental animals —Series V (a)

Group	First generation		Second generation		Third generation	
	Male	Female	Male	Female	Male	Female
	g.	g.	g.	g.	g.	g.
Ghee	13.55	8.19	13.62	9.37	13.43	9.78
Raw G. N. oil ..	14.83	8.23	13.65	8.88	14.28	9.89
Refined G. N. oil ..	15.00	9.28	12.78	9.18	12.79	9.08
Vanaspati, m.p., 37°C.	15.71	8.17	12.57	9.25	13.84	10.01
Vanaspati, m.p., 41°C.	16.13	8.98	14.01	8.69	12.72	9.18

Table 8: Average weekly increase in weight of experimental animals—Series VI: first generation

Group	Male	Female
	%	%
Ghee	5.20	6.59
Raw G. N. oil	4.66	1.95
Refined G. N. oil	3.50	2.10
Vanaspati, m. p., 37°C.	1.57	4.96
Vanaspati, m.p., 41°C.	4.30	3.27

The experiments [Series I to V(a)] were started early in March 1948. The first generation animals in all the 6 Series recorded a continuous increase in weight in the early stages and up to about the end of the eighth or ninth week. Thereafter the growth came to a standstill. The intense heat of the summer, which reache its maximum during this period, and the lack of cooling arrangements appear to be responsible for the arrest of the growth. The adverse effect of the hot season, although quite perceptible, appears to be comparatively less in the yeast supplemented Series III and all-supplemented Series V(a), as compared to others.

In the second and third generations, there was a continuous increase in weight over all the 12 weeks of experimental feeding. The third generation animals, despite the rigor of the summer heat, did not show static weights during any period, probably because of the cooling arrangement that was later provided.

REPRODUCTION AND LACTATION

The experimental rats, after 12 weeks' feeding on the experimental diet, were given a supplement consisting of 20 cc. skimmed milk and 5 g. of greens per rat per day. This feeding, which was started 4 weeks prior to mating, was continued during the gestation and lactation periods until the litters were 10 days old, when the greens were cut off and the milk continued until weaning time. In all the groups of Series I to V(a), half the number of males in every group was deprived of the supplement while all the females received the supplement. All the rats were serially numbered. The odd number males and all the females, both odd and even numbered, were given the liberal supplement. The even number males which were not getting the supplement were later slaughtered and the bone ash, tissue respiration and the R. B. C., W. B. C., and haemoglobin contents of the blood were determined.

After 4 weeks of liberal feeding, the animals were mated within the respective groups of the same series. The odd numbered males of the ghee group of the synthetic diet series were mated with the odd numbered females of the same group and the even numbered males with the even numbered females of the same group. Similarly, the odd numbered males of vanaspati, m.p., 37°C. group of the poor rice diet series were mated with the odd numbered females of the same group and the even numbered males with the even numbered females of the same group, and so on.

During the mating period, which extended over 3 weeks, each pair was kept in a separate cage. The even numbered males which were given neither milk nor greens were transferred to their own cages every morning when milk and greens were supplied to the females and were returned back to the cages in the afternoon after cleaning the cups of any bits of greens and milk. The results of reproduction and lactation studies of the experimental animals are presented in Tables 9 to 26.

Table 9: Reproductive and lactating capacities of experimental animals—Series I: first generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	1	3	1	2	6	21	5.8	5.8	1	16	30.5	29.8
Raw G. N. oil ..	2	3	2	1	18	5	4.8	5.4	16	4	24.8	29.3
Refined G. N. oil ..	3	3	2	1	17	8	5.0	5.2	15	6	26.7	29.7
Vanaspati, m.p., 37°C.	3	3	3	1	25	11	4.9	4.4	15	6	30.4	17.7
Vanaspati, m.p., 41°C.	3	2	3	1	18	9	4.7	5.5	15	6	32.7	21.9

Odd numbered females were mated with males receiving supplemented diet and even numbered females were mated with males receiving unsupplemented diet.

Table 10: Reproductive and lactating capacities of experimental animals—Series I: second generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	2	3	2	23	11	4.9	6.0	22	11	25.5	34.5
Raw G. N. oil ..	1	1	1	1	10	3	4.5	4.1	7	2	25.5	38.5

Table 11: Reproductive and lactating capacities of experimental animals—Series I: third generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	2	3	1	2	7	13	4.0	5.3	6	7	26.7	31.2

Table 12: Reproductive and lactating capacities of experimental animals—Series II: first generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	3	3	20	18	5.0	5.3	17	18	24.2	24.6
Raw G. N. oil ..	3	3	3	1	16	8	4.3	5.1	15	8	27.3	21.0
Refined G. N. oil ..	3	3	3	2	21	14	4.7	5.2	19	14	24.0	19.7
Vanaspati, m.p., 37°C.	3	3	3	3	21	19	4.9	4.7	18	18	24.8	24.6
Vanaspati, m.p., 41°C.	3	2	2	1	12	8	4.7	5.1	11	7	27.5	26.4

Table 13: Reproductive and lactating capacities of experimental animals—Series II: second generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	3	3	20	15	4.7	5.2	19	14	22.2	31.5
Raw G. N. oil ..	3	3	3	2	22	14	5.0	4.8	20	13	22.8	21.9
Refined G. N. oil ..	3	3	3	2	20	16	5.3	5.1	17	..	25.0	..
Vanaspati, m.p., 37°C.	3	3	3	3	23	22	4.6	4.9	23	20	19.0	21.0
Vanaspati, m.p., 41°C.	2	3	2	3	12	18	4.8	4.6	10	17	21.5	23.2

Table 14: Reproductive and lactating capacities of experimental animals—Series II: third generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litters g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	2	..	4	..	4.5	..	3	..	27.8	..
Raw G. N. oil ..	3	2	1	1	4	4	4.6	4.5	3	4	38.6	32.7
Refined G. N. oil ..	3	3	..	..	..	..	..	..	..	..	..	..
Vanaspati, m.p., 37°C.	3	3	1	..	3	..	5.0	..	3	..	27.3	..
Vanaspati, m.p., 41°C.	3	3	3	1	11	4	4.9	5.3	11	4	26.1	27.2

Table 15: Reproductive and lactating capacities of experimental animals—Series III: first generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	..	3	..	3	..	21	..	4.9	..	18	..	36.3
Raw G. N. oil ..	3	3	3	3	25	14	4.0	3.9	12	13	27.7	43.6
Refined G. N. oil ..	3	3	3	3	25	19	5.4	5.6	21	16	29.8	35.8
Vanaspati, m.p., 37°C.	3	3	3	3	23	25	4.0	5.2	13	21	42.9	31.7
Vanaspati, m.p., 41°C.	3	3	3	3	20	28	5.0	4.6	18	24	25.1	26.0

Table 16: Reproductive and lactating capacities of experimental animals—Series III: second generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	3	3	23	19	5.0	5.0	23	18	29.0	30.6
Raw G. N. oil ..	3	3	3	3	27	23	4.7	5.1	25	22	27.7	30.8
Refined G. N. oil ..	3	3	3	3	27	29	4.4	4.7	27	29	26.2	24.4
Vanaspati, m.p., 37°C.	3	3	3	3	26	26	4.8	4.6	24	26	29.8	25.4
Vanaspati, m.p., 41°C.	3	3	3	3	24	23	4.8	4.9	22	22	28.0	28.7

Table 17: Reproductive and lactating capacities of experimental animals—Series III: third generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	3	3	27	20	4.0	5.2	19	18	23.8	30.4
Raw G. N. oil ..	3	3	3	3	24	24	5.2	5.0	17	21	33.1	26.3
Refined G. N. oil ..	2	3	2	3	10	20	4.8	5.5	9	19	29.1	30.0
Vanaspati, m.p., 37°C.	3	3	3	3	26	22	4.7	5.4	22	18	22.9	31.1
Vanaspati, m.p., 41°C.	3	3	2	2	15	13	5.0	5.0	9	12	38.4	29.2

Table 18: Reproductive and lactating capacities of experimental animals—Series IV: first generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	3	2	24	14	4.1	4.6	20	10	16.7	24.5
Raw G. N. oil ..	3	3	3	1	20	6	4.1	5.2	10	6	33.7	27.0
Refined G. N. oil ..	3	3	2	2	15	14	5.4	5.5	15	4	27.3	20.6
Vanaspati, m.p., 37°C.	3	3	3	2	21	11	5.5	4.6	13	9	32.7	30.6
Vanaspati, m.p., 41°C.	3	3	3	2	23	14	4.8	5.1	19	14	28.2	24.1

Table 19: Reproductive and lactating capacities of experimental animals—Series IV: second generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	2	3	13	25	4.9	4.4	13	23	22.5	19.3
Raw G. N. oil ..	3	2	3	2	20	13	5.2	5.0	19	13	25.8	25.8
Refined G. N. oil ..	3	3	3	3	20	23	5.0	5.2	11	22	15.9	22.9
Vanaspati, m.p., 37°C.	3	3	3	3	21	19	4.3	5.3	19	19	20.1	24.7
Vanaspati, m.p., 41°C.	3	3	3	3	20	19	4.7	4.9	19	18	22.0	21.7

Table 20: Reproductive and lactating capacities of experimental animals—Series IV: third generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	3	2	17	7	4.9	4.7	16	6	20.1	25.0
Raw G. N. oil ..	3	3	3	2	14	6	5.1	5.1	15	5	24.3	24.3
Refined G. N. oil ..	1	3	1	1	4	8	4.8	5.1	3	8	35.8	21.6
Vanaspati, m.p., 37°C.	3	3	3	2	12	9	5.1	5.3	11	8	28.0	28.6
Vanaspati, m.p., 41°C.	3	3	3	2	16	9	4.4	5.2	16	8	23.4	25.8

Table 21: Reproductive and lactating capacities of experimental animals—Series V: first generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	3	1	22	8	4.7	4.8	18	6	32.1	36.7
Raw G. N. oil ..	3	3	3	1	24	9	5.0	5.0	19	9	32.5	20.0
Refined G. N. oil ..	3	3	3	2	23	18	5.2	5.6	20	18	35.1	22.1
Vanaspati, m.p., 37°C.	3	3	2	1	17	10	4.6	5.3	15	9	30.0	12.2
Vanaspati, m.p., 41°C.	3	3	3	1	20	5	4.5	4.9	17	4	34.2	44.0

Table 22: Reproductive and lactating capacities of experimental animals—Series V: second generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	3	3	24	28	4.3	4.3	24	25	24.3	25.4
Raw G. N. oil ..	3	3	3	3	26	26	4.6	5.0	25	26	22.6	24.2
Refined G. N. oil ..	3	3	3	3	26	22	4.7	5.2	22	22	23.1	28.1
Vanaspati, m.p., 37°C.	3	3	3	3	21	22	4.8	4.6	20	22	27.1	24.1
Vanaspati, m.p., 41°C.	3	3	3	3	20	23	4.8	4.8	20	23	25.1	29.7

Table 23: Reproductive and lactating capacities of experimental animals—Series V: third generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	3	3	14	8	4.6	4.8	13	4	24.7	26.7
Raw G. N. oil ..	3	3	3	..	8	..	4.8	..	8	..	25.0	..
Refined G. N. oil ..	3	3	1	1	4	3	4.6	4.8	3	3	32.8	29.1
Vanaspati, m.p., 37°C.	3	3	2	2	12	11	4.1	4.7	11	9	21.4	23.4
Vanaspati, m.p., 41°C.	3	3	3	1	16	7	4.5	4.4	13	6	25.5	19.6

Table 24: Reproductive and lactating capacities of experimental animals—Series V (a): first generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	2	3	2	27	13	4.8	6.0	26	10	32.8	41.8
Raw G. N. oil ..	3	3	2	3	11	25	5.0	5.2	6	21	55.3	33.7
Refined G. N. oil ..	3	3	2	3	18	29	5.4	5.4	16	27	33.0	28.9
Vanaspati, m.p., 37°C.	3	3	2	2	10	18	4.9	5.1	8	15	51.0	32.1
Vanaspati, m.p., 41°C.	3	3	3	3	20	18	5.2	5.0	19	13	34.6	35.4

Table 25: Reproductive and lactating capacities of experimental animals—Series V (a): second generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	3	3	18	23	5.3	5.2	15	16	34.4	29.6
Raw G. N. oil ..	3	3	3	3	26	25	5.3	5.4	26	23	30.3	30.0
Refined G. N. oil ..	3	3	3	2	28	18	5.2	5.0	21	17	27.9	31.0
Vanaspati, m.p., 37°C.	3	3	3	3	24	25	5.2	4.4	22	23	30.0	31.2
Vanaspati, m.p., 41°C.	3	3	3	3	29	18	4.8	5.3	27	17	26.4	37.2

Table 26: Reproductive and lactating capacities of experimental animals—Series V (a): third generation

Group	No. of females mated		No. of females giving litters		No. of young born		Av. wt. of each litter g.		No. surviving after 4 weeks		Av. wt. at weaning g.	
	odd	even	odd	even	odd	even	odd	even	odd	even	odd	even
Ghee	3	3	2	1	10	3	5.7	4.8	10	3	39.8	46.5
Raw G. N. oil ..	3	3	3	2	20	10	5.7	5.4	15	10	44.5	40.1
Refined G. N. oi. ..	3	3	2	2	11	12	5.5	5.0	10	10	39.3	34.4
Vanaspati, m.p., 37°C.	3	3	3	2	30	16	4.4	4.8	28	5	26.4	38.5
Vanaspati, m.p., 41°C.	3	3	2	3	18	24	4.7	4.7	12	19	32.5	32.4

First Generation

The reproduction and lactation records in the first generation were generally satisfactory.

The reproduction in the odd numbered females, *i.e.*, those mated with males on supplemented diet, was almost cent per cent successful and appeared to be slightly better than the results shown by the even numbers, *i.e.*, those mated with males receiving un-supplemented diet. In the ghee groups, however, only one even numbered female in each of the synthetic and poor rice diet + calcium carbonate Series and 2 in the poor rice diet + casein Series failed to reproduce. In all the other Series the reproduction in the ghee group was cent per cent.

There was some indication, however, that of all the 6 Series, the reproduction and lactation results in Series V (a), in which the diet was liberally supplemented, were the best.

Second Generation

In the second generation in the synthetic diet Series, the majority of the animals of the refined groundnut oil group and the vanaspati groups having died, the animals in these groups could not be mated. In the ghee group, out of the 6 females mated, 5 reproduced ; whereas in the raw groundnut oil group, out of the 2 females mated, none reproduced. The 2 animals were then given rest for a period of 2 weeks and during this period liberal feeding was continued. The animals were then mated a second time and the reproduction was successful. Thirteen litters were produced out of which 9 survived the weaning period. The experiment in the third generation was, therefore, continued with 9 rats only in the raw groundnut oil group.

The reproduction and lactation in other Series were satisfactory.

Third Generation

In the third generation, in the synthetic diet Series, 5 females of the ghee group were mated, of which 2 died during the mating period. The reproduction and lactation results of the remaining 3 were quite satisfactory and the weaning weight of the litters was almost the same as that obtained in the second generation.

In the poor rice diet Series when the animals were fed only on the unsupplemented diet (Series II), reproduction was poor and sterility was more common than in the animals of the second generation. In the other 4 Series, no material difference between the 2 generations was observed.

HEALTH AND MORTALITY OF THE ANIMALS

Synthetic Diet Series

First Generation

The animals generally maintained normal health. Signs of alopecia were visible in some of the animals. The signs continued for a few weeks and later disappeared. The animals in the ghee group were comparatively less affected. The condition of eye, paw and tail was normal throughout. From about the eighth week after starting experimental feeding a slight set-back in the rate of growth of animals, due probably to the summer heat, was observed. No deaths were recorded during the feeding period of 12 weeks. During the period of liberal feeding and mating some accidental deaths occurred in the ghee group. There were deaths in other groups too.

Second Generation

In the second generation, the animals of the synthetic diet series were adversely affected. The animals of the ghee group were the healthiest among all the series. Signs of alopecia were visible in one of the animals of the ghee group in the sixth week of experimental feeding which cleared up soon after. The rest of the animals of the ghee group showed no pathological symptoms.

In the raw groundnut oil group, 3 animals suffered from alopecia which appeared in the fourth or fifth week ; this cleared up in about 2 weeks. A few of the animals showed characteristic eye symptoms, e.g. dryness, by about the ninth or tenth week. This was followed gradually by a total closing of the eyes. Almost all the animals looked weak and stunted and developed a rough coat. Slight protrusion of the genital organs was observed in 2 animals.

As observed in the animals of the raw groundnut oil group, all the animals in the refined groundnut oil and the 2 vanaspati groups looked weak and stunted and had a rough coat. Some of them suffered from alopecia which cleared up soon after. Characteristic eye symptoms were observed by about the fifth week. In some cases extreme dryness of the eyes was noticed. In about 2 weeks after the appearance of the eye symptoms, in all cases, the eyes were almost completely closed. A number of animals suffered from diarrhoea. The condition of paw and tail of animals in all groups was normal.

The mortality in this series was extremely heavy in the second generation. One animal of the ghee group, 4 animals of the raw groundnut oil group and 10 each of the refined groundnut oil and vanaspati, m.p., 41°C. groups and all the 12 of vanaspati, m.p., 37 C. group died before completing 12 weeks of experimental feeding. Besides, 2 cases of respiratory troubles, acute congestion and inflammation of the bladder and retention of urine were observed in all the rest of the animals. Stones of the size of a pinhead were noticed in the bladders of some of the rats. Numerous small verminous cysts were seen in the livers of almost all the animals. Other organs were not apparently affected.

Animals receiving 60 I. U. of vitamin A daily showed symptoms very much resembling those seen in animals fed on vitamin A deficient diet. This observation is noteworthy. Later investigation of the livers of animals in this series revealed that sufficient vitamin A was present in the livers. It may be concluded that the symptoms were not due to vitamin A deficiency *per se*, but due to other causes whose nature is not fully clear.

Third Generation

As stated earlier, only animals of the ghee and the raw groundnut oil groups of the synthetic diet series could be pushed up to the group generation. In the synthetic diet series, the animals of the ghee third were generally healthy. Two of them showed signs of shedding the hair but these cleared up soon after. Only one female died during the experimental feeding period and post mortem examination gave no clue as to the cause of the death. In the last week of June, the weather became so intensely hot that in spite of all care 2 females died during the mating period. The animals of the raw groundnut oil group appeared to fare better in the third generation than in the second generation. Most of the animals of this group suffered from alopecia which in some cases was acute. The animals, however, showed none of the eye symptoms observed in the second generation, save one solitary case of dryness in the eye. It would appear that the liberal diet given to the second generation animals for a period of 4 weeks before remating, resulted in the building up of reserves in the mothers which were transmitted to the progeny.

Poor Rice Diets

First Generation

The animals of the first generation maintained normal health. There was a general set-back in the growth from about the eighth week of experimental feeding. This was due to the intense summer heat to which the animals were exposed in an enclosed verandah. The effect of heat on growth was least perceptible in Series III and V(a). Signs of alopecia were visible in some of the animals, particularly in the poor rice diet + calcium carbonate Series. In this Series too, the animals of the ghee group were less affected in comparison with animals of other fat groups. The falling off of hair continued for a few weeks and then cleared up. The condition of eye, paw and tail was normal throughout.

The number of deaths during the 12 weeks of feeding was only 4 out of a total of 360. No characteristic symptoms were observed during the post mortem examination of the dead rats. During the liberal feeding and mating period, a few more deaths were recorded, probably due to adverse climatic conditions and a few cases of pneumonia were observed.

Second Generation

The second generation animals of the poor rice diet Series, supplemented and unsupplemented, i.e. Series II to V(a), maintained on the whole normal health. Signs of shedding of hair were seen in some animals of Series II, IV and V(a) but this condition disappeared soon after it had made its appearance. The animals of the ghee group were least affected. The condition of eye, paw and tail of all animals was normal throughout. During the experimental feeding period of 12 weeks there were no deaths in any of the 5 Series except one which was due to an accident.

Third Generation

The third generation animals of the poor rice diet Series, supplemented and unsupplemented, i.e. Series II to V(a), like the first and second generations, maintained good health. Alopecia was observed in some anima but it cleared up in a short time. The condition of eye, paw and tail w-a normal throughout. Only 6 deaths were recorded during the experimental period of 12 weeks. Several deaths were recorded during the end of the liberal feeding or the beginning of the mating period when the weather became exceedingly hot and the improvised cooling arrangement provided little protection. Not only the experimental animals, but also a large number of stock rats died due to extreme heat during that period.

Poor Bengali Diet

The animals in this series were extremely weak. Alopecia was observed in most cases. Dryness of the eye was followed by the appearance of blood in the eye prior to death in most cases.

All the males of the vanaspati, m.p., 37°C. group and all the females of the refined groundnutoil group died before the end of the fourteenth and nineteenth weeks respectively of experimental feeding. The lowest mortality was observed in the animals of the ghee group. In most cases deaths were due to the congestion of lungs. In a few cases the bladder was found to be distended due to urine retention. Ulcerated gastritis accounted for death in a few cases.

TISSUE RESPIRATION, ASH CONTENT AND BLOOD ANALYSIS

Measurement of tissue respiration in the Warburg apparatus provides valuable information on the state of activity of any particular organ. The oxygen consumption of a tissue is an indication of the state of cell metabolism and comparative studies of tissue respiration in different groups of animals help in the understanding of the effect of feed, management and other variables on the integrity of the tissue cells. For ascertaining the effect of various fats on the health of rats, it was felt that such studies on tissues of third generation animals in the different groups might provide useful data. Histological examination had revealed pathological changes due to the continued ingestion of different fats, and it was reasonable to expect that if the structural integrity of tissues was affected, respiratory rates would likewise be affected. Further, if differences in respiratory rates were observed, it should be possible to investigate further and account for such differences quantitatively in relation to gross tissue destruction or lack of specific enzymes and coenzymes. The liver tissue was selected for the study, as the liver is one of the very first organs to be affected by dietary changes ; also, the oxygen consumption of liver tissue slices is not dependent on the addition of extra glucose substrate and the inherent respiratory activity of the tissue can be correctly ascertained.

The results obtained are given in Table 27. As our purpose was mainly to compare one group of animals with another group, all respiratory measurements were carried out in air instead of pure oxygen. Triplicate measurements were carried out for each sample, and the average of four 30-minute periods for each Warburg vessel was taken. For any sample the variation among triplicate measurements usually lay within 10 per cent. Any value lying beyond + 20 per cent of the medial value was rejected. The bath temperature was maintained at 37.5° ± 0.2°C. Usually 3 rats from each group were examined.

Table 27: Respiratory coefficients of liver slices of experimental animals in different fat groups—third generation

Group	Series II	Series III	Series IV	Series V	Series V(a)
Ghee	1.23	1.83	1.46	2.00	1.83
Raw G. N. oil ..	1.26	1.72	1.25	1.17	1.96
Refined G. N. oil ..	1.29	1.73	0.72	1.65	1.81
Vanaspati, m.p., 37°C.	1.39	1.84	0.79	1.13	1.15
Vanaspati, m.p., 41°C.	1.45	1.66	0.87	1.18	2.04

Ash content of bones—Moisture and fat-free bones of experimental animals of the third generation were ashed in a muffle furnace. The figures obtained were converted into percentages of moisture and fat-free bones. The results are presented in Table 28.

Table 28: Percentage of bone ash of experimental rats—third generation

Group	Series II	Series III	Series IV	Series V	Series V(a)
Ghee	55.0	59.7	62.1	58.0	65.0
Raw G. N. oil ..	53.1	60.1	59.0	58.4	64.0
Refined G. N. oil ..	51.1	62.3	58.1	55.2	66.0
Vanaspati, m.p., 37°C.	53.1	58.5	60.0	55.1	64.3
Vanaspati, m.p., 41°C.	54.3	61.2	56.5	55.8	63.2

Blood Analysis—The blood of experimental animals of the third generation was analysed for R.B.C., W.B.C. and haemoglobin contents and differential leucocyte count. The results are given in Table 29.

It will beseen that there was considerable variation in the values within the same series and among different series, and no definite conclusion could be drawn.

Table 29 : R.B.C., W.B.C. and haemoglobin content and differential leucocytes count of blood of experimental animals—third generation.

	R.B.C. millions c.mm.	W.B.C. thousands c.mm.	Haemo-globin 100 cc.	Differential count (%) Polymorph neutrophills	Lymphocytes	Monocytes	Eosinophills	Basophills
Series I								
Ghee	7.22	6.2	12.00	45	47	6	2	..
Raw G.N. oil	6.52	6.3	9.20	23	72	4	1	..
Refined G.N. oil ..	..	..	..	..	..	..	..	..
Vanaspati, m.p., 37°C.	..	..	..	..	..	..	..	..
Vanaspati, m.p., 41°C. ..	..	..	..	..	..	..	..	..
Series II								
Ghee	8.60	7.3	12.25	..	..	..	..	..
Raw G.N. oil	7.47	9.5	10.90	26	65	8	1	..
Refined G.N. oil ..	8.36	11.1	11.49	52	40	7	1	..
Vanaspati, m.p., 37°C.	7.74	8.6	10.44	43	45	8	1	..
Vanaspati, m.p., 41°C.	7.59	7.8	11.25	40	49	9	2	..
Series III								
Ghee	9.78	13.7	14.13	43	47	8	2	..
Raw G.N. oil	9.55	13.4	13.73	34	54	10	2	..
Refined G.N. oil ..	9.39	14.4	13.37	23	66	8	3	..
Vanaspati, m.p., 37°C.	9.02	10.2	14.00	23	61	13	3	..
Vanaspati, m.p., 41°C.	9.31	9.0	12.70	21	62	17	..	..
Series IV								
Ghee	8.17	9.3	11.60	41	48	9	2	..
Raw G.N. oil ..	9.29	11.9	11.20	47	30	20	3	..
Refined G.N. oil ..	7.26	7.3	10.97	36	58	4	3	..
Vanaspati, m.p., 37°C.	7.58	8.0	11.10	29	59	11	1	..
Vanaspati, m.p., 41°C.	7.22	8.5	12.80	42	48	9	1	..
Series V								
Ghee	7.51	9.0	12.50	40	53	6	1	..
Raw G. N. oil	7.31	5.6	13.05	36	55	8	1	..
Refined G.N. oil ..	8.13	6.4	12.80	40	46	12	2	..
Vanaspati, m.p., 37°C.	7.08	6.4	11.83	44	45	9	2	..
Vanaspati, m.p., 41°C.	7.03	7.4	11.53	34	60	5	1	..
Series V (a)								
Ghee	9.03	11.0	14.13	31	60	8	1	..
Raw G.N. oil	8.26	8.9	12.33	26	68	5	1	..
Refined G.N. oil ..	9.65	10.1	12.00	27	51	10	2	..
Vanaspati m.p., 37°C.	8.00	8.2	11.37	30	64	5	1	..

SECTION IV

Animal Experiments carried out at the University College of Science and Technology, Calcutta*

The following series of experiments were conducted:

Series I Synthetic diet
Series II Poor rice diet
Series III Poor rice diet × yeast × vitamins A, D and E
Series IV Poor rice diet × calcium carbonate
Series V Poor rice diet × casein
Series VI Poor Bengali diet A
Series VII Poor Bengali diet B (cooked)

PREPARATION OF DIETS AND FEEDING

The poor rice diet was prepared by cooking the required quantities of rice, dal, minced leafy and non-leafy vegetables, salt and heated oil or fat with water in a boiling water bath till the diet became sufficiently soft. It was found that the mixing of 350 cc. of water with 120 g. of the mixture of food ingredients and 35-40 minutes' cooking gave a soft mass of food. The food for one group with a particular oil or fat was cooked in one lot and then distributed among the 12 rats of this group. In addition, 0.9 cc. of a 10 per cent suspension of Klim milk powder was given in a separate dish daily to every rat. This was done in order to ensure the consumption of all the milk by each rat irrespective of the amount of diet consumed. Care was taken to see that sufficient cooked food was given to each rat and that the pot always contained some remnants on the following day to ensure the supply of diet *ad libitum*.

For Series III (poor rice diet supplemented by yeast and vitamins), Squibb's yeast powder (4 %) was mixed with the cooked diet thoroughly along with oil or fat while the diet was still warm. α-Tocopherol was incorporated in the vitamins A and D concentrate solution in propylene glycol and the solution was always given in a separate dish. Each rat received daily 0.5 mg. of α-tocopherol, 60 I.U. of vitamin A and 10 I.U. of vitamin D.

In Series IV and V (poor rice diet supplemented by calcium carbonate and casein respectively), the diets were cooked with the supplements in the same way as the poor rice diet alone.

Poor Bengali diet A was prepared in the following way: The required quantity of rice, after being washed, was mixed with powdered dal, mung

*The work described in this section was carried out under the supervision of Dr. B. C. Guha, formerly Chief Technical Adviser to the Food Department, Delhi, and now member of the Damodar Valley Corporation. The experimental work was carried out by Messrs. P. N. Sen Gupta, S. K. Roy, A. N. Bose, M. N. Roy, S. Das Gupta and B. Chatterjee.

and *masur*, minced leafy and other vegetables, salt and beaten egg. The mixture was cooked on a boiling water bath till sufficiently soft. In a separate beaker, fish and meat were boiled with water till sufficiently soft, minced and incorporated in the cooked diet along with the test oil or fat (heated previously to 180°C. for 5 minutes). The diet was then thoroughly mixed.

The diets so prepared were fed to the experimental rats. At the end of the eighth week of the experimental period, the animals were given per head per day an extra 5 g. of green vegetables and 20 cc. of 10 per cent solution of Selo brand skimmed milk powder (*Central Farm Products*, U. S. A.) containing not more than 1.25 per cent butterfat on dry basis. The administration of these supplements was continued till the litters were 10 days old, after which the green vegetables were discontinued. The addition of 20 cc. of skimmed milk was continued till the end of the weaning period when the young were put on to the second generation experiment.

The young animals in the second generation experiment were not given any more milk and were fed on the poor Bengali diet. It was observed that the animals, irrespective of the fat in the diet, became emaciated and it was apprehended that they might die before reaching the ninth week of the experimental period, when greens and milk would be given. Therefore, 5 cc. of 10 per cent skimmed milk powder suspension were given to the rats, irrespective of the groups to which they belonged, immediately weaning and throughout the experimental period.

Poor Bengali diet B was prepared by cooking rice with pulse and vegetables, to which boiled fish was added. Before serving out, 5 per cent of the test oil or fat was added to the cooked mass and the whole mixed thoroughly. It was suggested by the Izatnagar workers that only fish should be cooked and other ingredients should be supplied raw. However, when this suggestion was received, the experiments using the cooked diet were already well in progress both in this and in other laboratories. Since there was some reference to the harmful effects of raw *arhar* pulse and since cooked diet is generally consumed in the country, it was thought unnecessary at this stage to change from the cooked to the raw after diet.

The synthetic diet was prepared by mixing the finely powdered ingredients into a uniform mass. About a week's ration was prepared at a time. The daily requirements were weighed out into 5 different beakers for mixing with the test oil or fat.

EXPERIMENTAL PROCEDURE

In the first generation, each series consisting of 60 rats was divided into 5 groups of 12 rats each. Littermates from the stock breeding colony, soon after weaning, were selected and distributed in the different groups. The average weight of rats in the groups varied from 30 to 35 g. at the start of the experiments. The rats were maintained in separate cages and were regularly fed with the different diets according to schedule. Milk and vitamin supplements, where necessary, were administered separately.

Experimental feeding was continued for 12 weeks except in the case of the poor Bengali diet B, which was continued for 26 weeks.

During the experimental period the rats were examined for signs of alopecia, roughness of the skin, defects in the eye, etc. The weights were recorded weekly. Daily food consumption was recorded in the earlier stages of the investigation but was subsequently discontinued.

The rats were paired for mating after 12 weeks on the experimental diet. The same diet was continued throughout. After about 3 or 4 weeks when signs of pregnancy were apparent, the females were separated from the males. A record of the young born, their number and survival was maintained.

For 4 weeks the young were kept with the mothers, after which they were separated and maintained in individual cages on their respective experimental diets.

The second generation experiments were continued with Series II, III and IV. Experiments on the poor Bengali diet A rats were also continued up to the second generation. Thirty-six rats of the second generation experiments receiving poor Bengali diet A were fed on poor Bengali diet B after they had already been fed on poor Bengali diet A for 8 weeks. Besides these, another set of 29 rats which were bred from the first generation rats receiving poor Bengali diet A were put on to diet B immediately after weaning. In doing so, littermates were distributed as evenly as possible among the different groups.

Experiments on the first generation only were carried out in Series I and Series V.

FAT AND VITAMIN A CONTENT OF RAT LIVERS

For the estimation of fat and vitamin A in the liver, the method recommended by the *Association of Vitamin Chemists, Inc.* was adopted. The liver was quickly taken out after killing the animals by a blow on the head. About 3 g. of the liver were pulped, dried with anhydrous sodium sulphate in a vacuum desiccator and extracted in soxhlet with petroleum ether for 5 hours to obtain the fat. Another aliquot of 1 g. was dried in an air-oven at 105°C. for 24 hours to obtain the moisture content of the tissue. The fat was saponified and the non-saponifiable portion extracted with ether. The ether extract was evaporated and the residue taken in chloroform for the estimation of vitamin A by the antimony trichloride method in a Lovibond Tintometer.

GENERAL REMARKS

The daily and weekly observations on the condition of health of the rats may be summarised as follows:

In Series I, i.e. in experiments with the synthetic diet, the performance of the rats was very good for the first 6 to 8 weeks. Subsequently, however, there was a fall in the rate of growth in all the groups and some of the rats developed symptoms of diarrhoea. Apparently there was some infection. The diseased rats were segregated and gradually nearly 70 per cent of them recovered, while the rest died. Apart from this, the rats did not show any other untoward symptoms.

As regards the condition of the rats of Series II on poor rice diet during

the period of 12 weeks, no rat was found to be diseased ; the rats did not grow well and some of them did not have a glossy coat. The maximum weight of the male and female rats did not exceed 90 g. and the minimum weight was as low as 60 g. after 12 weeks of feeding on the diet. This shows that nutritionally the diet was very poor. The condition of the animals did not show any difference whether the food was cooked with oil, vanaspati or ghee.

During the experimental period of 12 weeks in Series III (poor rice diet supplemented by yeast and vitamins A, D and E), many of the rats were healthy and grew well. Falling of hair for 2-3 weeks was noticed in the case of only 2 rats ; after 2-3 weeks, the hair grew again. The growth of one rat (Group 5 — ghee, female) was satisfactory for 2 weeks and then the weight declined gradually and the rat died at the end of the fifth week. Another female rat of the same group grew normally up to the second week but died in the third week. All other rats were healthy and they had a glossy coat. The maximum weight attained was 176 g. and the minimum weight was 93 g.

The growth of rats in Series IV (poor rice diet supplemented by calcium carbonate) was not as satisfactory as that of rats in Series III (poor rice diet supplemented by yeast and vitamins) ; yet the growth was better than that of the rats in Series II (unsupplemented poor rice diet). Excepting partial shedding of hair in some rats, no other symptom was observed. Even this shedding of hair lasted for a short period only, after which fresh hair grew in all cases. The maximum weight rose to 150 g. and the minimum weight was 75 g. The condition of rats in the different groups of the Series was more or less the same whether they were fed on oil, vanaspati or ghee.

The rats in the different groups of Series V (poor rice diet supplemented with 7 per cent casein) showed similar rates of growth during the experimental period. The general condition of health was satisfactory.

The growth of rats in all the groups of Series VI (poor Bengali diet A) was unsatisfactory during the early period of the experiment ; the growth was comparable with that of rats fed on unsupplemented poor rice diet. The rats were emaciated. According to the programme approved by the Committee, 20 cc. of skimmed milk and 5 g. of green vegetables were given to each rat at the end of the eighth week of the experimental period. These supplements produced a salutary effect on growth. In several cases, the increase in weight per week rose to 15 g. The rats looked healthy, had a glossy coat and there was no sign of deficiency disease at the end of the experimental period. The litters born to these rats appeared to be healthy. The mortality of the young born was negligible and cannibalism was not noticed.

The rats fed on the modified Bengali diet B maintained a steady increase in weight and for the first 12 weeks the growth was comparable with that of rats fed on poor Bengali diet A. The general condition of the rats appeared to be satisfactory throughout the period. There was no noticeable shedding of hair or roughness of coat though from time to time falling of hair was observed in some individual rats. No coot of blindness was observed in any of the animals even after prolonged feeding for 28 weeks. There was no indication of any abnormal mortality. No attempt,

however, was made to study the reproductive and lactating capacities of these rats.

Reproduction and Lactation

The rats in Series II (poor rice diet) did not reproduce well, and the survival of young was unsatisfactory. This was true irrespective of the fat component in the diet. In Series III, reproduction and survival of young were satisfactory. Reproduction in the rats of Series IV was unsatisfactory. Reproduction and survival of young were satisfactory in the groups fed on Bengali diet A. Here also the results were similar in all the different fat groups.

RESULTS

The results of experiments are summarised in Tables* 1—16.

Table 1: Average weekly increase in weight of experimental animals—Series I, first generation

Group	Male	Female
	g.	g.
Ghee	8.1	8.0
Raw G. N. oil.	7.0	7.7
Refined G. N. oil	6.8	6.9
Vanaspati, m.p., 37°C.	7.3	6.8
Vanaspati, m.p., 41°C.	8.4	7.8

Table 2: Average weekly increase in weight of experimental animals—Series II, first and second generations

Group	First generation		Second generation	
	Male	Female	Male	Female
	g.	g.	g.	g.
Ghee	2.6	2.4	4.4	4.4
Raw G. N. oil	3.2	2.6	..	..
Refined G. N. oil	2.0	2.6	..	..
Vanaspati, m.p., 37°C. ..	3.0	3.4	4.4	4.1
Vanaspati, m.p., 41°C. ..	2.7	2.2	..	..

Table 3: Average weekly increase in weight of experimental animals—Series III, first and second generations

Group	First generation		Second generation	
	Male	Female	Male	Female
	g.	g.	g.	g.
Ghee	9.8	6.0	5.0	4.7
Raw G. N. oil	5.8	5.0	5.8	4.6
Refined G. N. oil	6.4	6.1	6.5	5.5
Vanaspati, m.p., 37°C. ..	8.1	6.2	6.1	5.6
Vanaspati, m.p., 41°C. ..	6.3	5.8	5.9	4.9

* In these Tables only the average figures have been given. The data for individual animals were utilised for statistical analyses.

Table 4: Average weekly increase in weight of experimental animals—Series IV, first and second generations

Group	First generation		Second generation	
	Male	Female	Male	Female
	g.	g.	g.	g.
Ghee	7.1	5.4	5.7	4.4
Raw G. N. oil	5.0	4.8	..	..
Refined G. N. oil	6.3	5.2	..	5.4
Vanaspati, m.p., 37°C. ..	6.3	4.7	6.0	5.1
Vanaspati, m.p., 41°C. ..	8.2	4.6	6.4	5.6

Table 5: Average weekly increase in weight of experimental animals—Series V, first generation

Group	Male	Female
	g.	g.
Ghee	7.5	7.3
Raw G. N. oil	5.7	5.3
Refined G. N. oil	5.5	5.9
Vanaspati, m.p., 37°C.	6.2	6.6
Vanaspati, m.p., 41°C.	6.7	6.9

Table 6: Average weekly increase in weight of experimental animals—Series VI: first and second generations

Group	First generation		Second generation	
	Male	Female	Male	Female
	g.	g.	g.	g.
Ghee	6.2	5.1	5.7	4.9
Raw G. N. oil	6.6	5.7	5.5	5.4
Refined G. N. oil	6.0	5.0	5.6	4.9
Vanaspati, m.p., 37°C. ..	5.8	5.9	6.3	5.5
Vanaspati, m.p., 41°C. ..	6.3	5.5	4.9	5.2

Table 7: Average weekly increase in weight of experimental animals—Series VII, first generation

Group	Av. for 12 weeks		Av. for 25 weeks	
	Male	Female	Male	Female
	g.	g.	g.	g.
Ghee	8.1	5.8	6.3	4.5
Raw G. N. oil	6.6	5.8	4.7	3.8
Refined G. N. oil	6.7	5.6	4.6	4.1
Vanaspati, m.p., 37°C. ..	8.3	5.7	6.2	4.4
Vanaspati, m.p., 41°C. ..	6.6	6.3	5.0	4.9

Table 8: Reproductive and lactating capacities of experimental animals—Series II, first generation

Group	No. of female rats	No giving birth to young ones	No. of young ones born	No. of young ones surviving
Ghee	6	6	22	2
Raw G. N. oil	6	2	8	..
Refined G. N. oil	6	..	..	..
Vanaspati, m.p., 37°C. ..	6	4	14	3
Vanaspati, m.p., 41°C. ..	6	2	6	..

Table 9: Reproductive and lactating capacities of experimental animals—Series III, first generation

Group	No. of female rats	No. giving birth to young ones	No. of young ones born	No. of young ones surviving
Ghee..	4	1	8	8
Raw G. N. oil	6	4	25	6
Refined G. N. oil	6	5	33	15
Vanaspati, m.p., 37°C. ..	5	4	34	9
Vanaspati, m.p., 41°C. ..	6	6	35	6

Table 10: Reproductive and lactating capacities of experimental animals—Series IV, first generation

Group	No. of female rats	No. giving birth to young ones	No. of young ones born	No. of young ones surviving
Ghee..	6	6	35	9
Raw G. N. oil	5	2	11	6
Refined G. N. oil	5	4	16	3
Vanaspati, m.p., 37°C. ..	6	2	13	5
Vanaspati, m.p., 41°C. ..	6	4	18	5

Table 11: Reproductive and lactating capacities of experimental animals—Series VI, first generation

Group	No. of female rats	No. giving birth to young ones	No. of young ones born	No. of young ones surviving
Ghee..	4	2	15	11
Raw G. N. oil	4	4	17	16
Refined G, N, oil	4	4	23	22
Vanaspati, m.p., 37°C. ..	4	2	13	13
Vanaspati, m.p., 41°C. ..	4	4	20	17

Table 12: Vitamin A and fat content of livers of experimental animals—Series I (feeding period, 12 weeks)

Group	No. of rats	Av. fresh liver wt. g.	Av. moisture %	Av. fat %	Av. vitamin A I. U./g. dry wt.
Ghee	6	4.0	68.8	3.0	176.3
Raw G. N. oil	6	2.9	70.1	2.8	343.7
Refined G. N. oil	6	3.0	66.7	2.2	251.2
Vanaspati, m.p., 37°C.	6	3.1	68.2	3.0	301.0
Vanaspati, m.p., 41°C.	6	3.3	68.1	2.8	206.3

Table 13: Vitamin A and fat content of livers of experimental animals—Series II (feeding period, *c.* 1 year)

Group	No. of rats	Av. fresh liver wt. g.	Av. moisture %	Av. fat %	Av. vitamin I.U./g. dry wt.
Ghee	6		66.2	5.1	460.3
Vanaspati, m.p., 37°C.	5	4.7	70.9	3.4	190.3
Vanaspati, m.p., 41°C.	5	5.1	69.3	4.1	140.5

Table 14: Vitamin A and fat content of livers of experimental animals—Series III (feeding period *c.* 1 year)

Group	No. of rats	Av. fresh liver wt. g.	Av. moisture %	Av. fat %	Av. vitamin I.U./g. dry wt.
Ghee	6	4.9	69.6	2.4	262.0
Refined G.N. oil ..	6	5.3	67.4	2.8	203.2
Vanaspati, m.p., 37°C.	4	5.6	66.1	2.3	200.3
Vanaspati, m.p., 41°C.	4	4.6	67.3	2.3	299.5

Table 15: Vitamin A and fat content of livers of experimental animals—Series IV (feeding period, *c.* 1 year)

Group	No. of rats	Av. fresh liver wt. g.	Av. moisture %	Av. fat %	Av. vitamin A I.U./g. dry wt.
Ghee	6	5.1	57.6	2.6	227.0
Refined G.N. oil ..	6	5.9	68.4	2.6	317.0
Vanaspati,m.p., 37°C.	5	4.9	69.1	2.5	269.6

Table 16: Vitamin A and fat content of livers of experimental animals—Series VI (feeding period, 12 weeks)

Group	No. of rats	Av. fresh liver wt. g.	Av. moisture %	Av. fat %	Av. vitamin A I.U./g. dry wt.
Ghee	6	4.2	68.5	2.3	310.8
Raw G.N. oil.. ..	6	5.0	68.5	2.5	197.0
Refined G.N. oil	6	3.8	69.4	1.9	145.0
Vanaspati, m.p., 37°C.	6	5.2	68.5	2.6	138.2
Vanaspati, m.p., 41°C.	6	4.3	66.2	2.9	238.7

HUMAN METABOLISM EXPERIMENTS

SECTION I

Experiments carried out at the Indian Institute of Science, Bangalore*

A—Utilisation of ingested fats and levels of plasmalipoids

The digestibility of fats and oils in experimental animals and in human beings has been extensively investigated[1—11]. The general trend of evidence is that most of the commonly used animal and vegetable fats, including some hydrogenated fats, are digested to essentially the same degree. Conflicting results, however, have been reported with regard to the apparent relationship between the melting points and the digestibility coefficients of edible fats. Langworthy and Holmes[1], Holmes[2] and Langworthy[3] showed that the digestibility coefficent of fats with melting points above the body temperature (37 °C.) varied inversely as the melting point, while Deuel and Holmes[4] found that partially hydrogenated vegetable oils and blended hydrogenated fats with melting points below 50°C. were completely digested. Lower values for the digestibility of fats having melting points of 50 °C. and above, viz., mutton fat[1], oleostearin[12], deer fat[5], and almost completely hydrogenated fats[4] have been reported. Comparing the relative digestibilities of lards, hydrogenated shortenings and various other fats, Hoagland and Snider[13,14] found no consistent relationship between digestive coefficients and melting points of fats in the ranges of 26° to 47°C. and 39° to 56°C.

A distinct fall in the degree of unsaturation of serum fatty acids and in the concentration of cholesterol, total fatty acids and total lipoids in the blood of rats, goats, dogs, calves and of adult human subjects on an extremely low fat regimen has been consistently observed[12—21]; Knudson[22], Cook[23] and Eckstein[24] found that food cholesterol is not efficiently absorbed unless fat also was present in the diet in optimal concentrations. The observation that the amount of dietary fat determines the level of serum cholesterol, however, has been questioned by Curtis *et al.*[25] and Man and Peters[26]. A tendency for the iodine number of serum lipoids to vary directly and for the total amount of serum lipoids to vary inversely with the iodine number of dietary fat has been observed[27, 18, 23]. Nhavi and Patwardhan[29] found no statistically significant relationship between the level of fat intake, the total amount of serum lipoids and the iodine number of serum fatty acids in normal human subjects.

* The work described in this section was carried out by Drs. S. M. Bose and C. R. Krishnamurthy under the supervision of Dr. V. Subrahmanyan. Mr. A. Ramaswamy assisted in drawing blood samples and recording health observations.

In the present section are recorded the results obtained on the comparative utilisation of raw and refined groundnut oils, two vanaspatis (m.p., 37° and 41°C.) made from them and ghee in 12 adult human volunteers and the influence of these fats on the level and composition of plasma lipoids.

EXPERIMENTAL PROCEDURE AND RESULTS

Twelve healthy adults who carried out ordinary duties in the laboratories were selected for the experiments. They were kept under strict supervision during the experimental period.

The diet used was the poor rice diet, the composition of which has already been given under Animal Experiments.

Fat at the rate of 50 g. per head per day was given to the subjects and other dietary items were calculated on this basis. To make the food tasty, spices, tamarind and chillies were used in small amounts as is the usual practice in South India.

The oils and vanaspatis used in these experiments were obtained from the *Hindustan Vanaspati Manufacturing Co., Ltd.*, Bombay, and the ghee from the Indian Dairy Research Institute, Bangalore.

"Upma", a common South Indian preparation of rice flour, and a cup of coffee were served to each person during both breakfast and tea time. Cooked rice with "Sambar" (a preparation of dal, vegetables and a small amount of tamarind) and vegetable curry were served during lunch and dinner.

The major portion of the oil or fat was used up in the upma preparation. The total quantity of upma prepared in the morning as well as in the afternoon was distributed equally among the subjects. The remaining portion of the oil or fat was consumed along with sambar and curry during lunch and dinner. "Sambar" and curry were also served equally. Cooked rice was served *ad lib.* Buttermilk was prepared from half the total amount of whole milk powder allotted for the subjects per day and was given to them equally during lunch. From the other half, milk was prepared and this was served equally along with coffee in the morning and in the afternoon.

For each of the first two Series of experiments on raw and refined groundnut oils, the duration of the experiment was 17 days, the first seven days being observed as the preliminary period and the last ten days (two periods of five days each) as the experimental period when records of the daily food intake were kept and urine and faeces were collected. As no significant difference could be traced between the results for two 5-day periods, the duration of the experiment in the subsequent series was re-

duced to 12 days, the first seven days being counted, as before, as the preliminary period and the following five days only as the experimental period.

At the end of the experimental period of each Series, 4-5 cc. of blood were drawn from the vein of each subject before breakfast into test tubes containing a few crystals of sodium citrate and immediately centrifuged. The total cholesterol and the total fatty acids in the plasma were determined by Bloor's method[30]. For the determination of the iodine number of plasma fatty acids, Yasuda's method[31] was followed. Plasma lipoid analysis was carried out twice during the experimental period, only in the first series. In some cases plasma analysis could not be carried out owing to failure in drawing adequate quantity of blood.

The urine samples were collected for 24-hour periods in bottles containing 50 cc. of conc. sulphuric acid, made up to a suitable volume each day and an aliquot portion was preserved under toluene in the cold room for 5-day periods, at the end of which they were analysed for calcium, phosphorus and nitrogen.

The subjects were given carmine in gelatin capsules for marking the faeces during the experimental period. The faeces excreted during the 24-hour period were individually collected in enamelled pans and preserved up to the time of drying with 25 cc. of absolute alcohol containing 1.0 per cent conc. sulphuric acid and 1.0 per cent carbolic acid. The wet faeces of each subject was weighed every day and thoroughly mixed by stirring with an enamelled ladle. Half the quantity was spread in thin layers in enamelled dishes, dried in a current of hot air (temp., *c.* 44°C.) in mechanical driers, collected individually in stoppered bottles for 5-day periods, weighed at the end of each period, powdered, preserved in the cold room and analysed for total and split fat content by the Second Soxhlet method of Harrison[32], and for calcium, phosphorus and nitrogen.

During the experimental period in each Series, a specimen of the cooked ration (excluding rice) representing the average consumption per subject per day was dried on the water bath and analysed for fat, calcium, phosphorus and nitrogen. Rice was analysed separately. The calcium content of drinking water was found to be practically negligible. The figures for the average daily intake of fat, calcium, phosphorus and nitrogen for individual subjects in each Series were calculated.

A control experiment on the same subjects to determine endogenous excretion of fat and to study the utilisation of calcium, phosphorus and nitrogen in the basal, practically fat-free ration was also carried out. The basal ration (excluding the fat supplement) consumed per subject per day contained on the average 6.83 g. of fat.

The results relating to fat metabolism and plasma lipoid analysis for individual subjects are given in Tables 2 to 6. The average daily consumption of food by each subject when receiving different fat supplements as well as the average corresponding excretion of faeces are indicated in Table 1.

Table 1: Average daily intake of food and excretion of faeces

Subject No.	Food (dry wt. in g.)								Faeces (dry wt. in g.)							
	B	C_1	C_2	R_1	R_2	V_1*	V_2*	G*	B	C_1	C_2	R_1	R_2	V_1*	V_2*	G*
1	n.a	645	625	655	655	670	n.a.	n.a.	n.a.	27.5	29.9	34.2	27.0	27.9	n.a.	n.a.
2	n.a.	780	775	n.a.	n.a.	n.a.	n.a.	n..a	n.a.	39.6	34.4	n.a.	n.a.	n.a.	n.a.	n.a.
2A	725	n.a	n.a	778	770	762	778	786	49.1	n.a	n.a	31.3	35.7	47.0	56.0	52.3
3	698	744	740	748	748	750	756	764	32.3	25.4	26.2	25.1	26.2	32.0	38.4	37.2
4	762	815	820	830	n.a.	820	858	852	44.6	38.4	42.8	26.7	n.a.	35.6	49.3	35.6
5	748	790	786	792	792	786	782	780	39.4	47.7	56.8	34.5	41.8	49.9	47.8	32.4
6	762	806	812	820	812	826	818	828	39.2	46.1	53.9	45.4	55.8	69.7	55.6	54.8
7	837	880	880	890	885	885	892	882	47.4	47.2	56.6	46.8	36.9	46.5	42.5	37.5
8	739	775	782	782	793	793	798	790	30.7	38.6	29.8	30.8	32.3	29.2	57.9	30.3
9	615	655	655	640	640	665	n.a.	670	28.0	26.7	33.9	25.8	27.9	26.3	n.a.	35.4
10	794	840	848	845	845	852	865	865	56.5	54.2	45.4	33.1	49.6	52.5	54.0	35.3
11	794	870	880	870	850	n.a.	n.a.	895	82.9	50.2	72.5	57.3	45.9	n.a.	n.a.	57.9
12	837	890	890	882	888	888	908	900	77.1	72.4	66.0	54.7	49.0	86.1	85.5	75.6

B. Basal (fat-free) diet — control; C_1, Raw G. N. oil: first period of metabolism; C_2, Raw G. N. oil: second period of metabolism; R_1, Refined G. N. oil: first period of metabolism; R_2, Refined G. N. oil: second period of metabolism; V_1, Vanaspati, m.p., 37°C.; V_2, Vanaspati, m.p., 41°C.; G, Cow ghee; n.a., Subjects could not join owing to reasons unconnected with the experiments.

* Results relate to the entire period under experiment.

Table 2: Comparative utilisation of raw and refined groundnut oils, vanaspatis and ghee

Diet	Av. daily fat intake	Av. daily fat excretion.	Av. daily fat excretion due to added fat	% Absorption of added fat	% Absorption in dried faeces	% faecal fat: split fat	% faecal fat: unsplit fat
	g.	g.	g.				
Subject No. 1 ; 22 yrs. ; wt., 44.8 kg.							
C_1	56.45	6.68	..	..	24.28	72.55	27.45
C_2	56.45	6.60	..	..	22.10	79.21	20.79
R_1	56.50	6.44	..	..	18.83	76.10	23.90
R_2	56.50	6.51	..	..	24.15	82.04	17.96
V_1	56.48	7.22	..	..	25.88	70.18	29.82
Subject No. 2 ; 24 yrs. ; wt., 42.4 kg.							
C_1	57.06	6.40	..	..	16.15	70.1	29.85
C_2	57.06	6.28	..	..	18.23	78.02	21.98
Subject No. 2A ; 25 yrs. ; wt., 46.8 kg.							
B	6.83	3.02	..	..	6.15	50.08	49.92
R_1	56.90	5.07	2.05	95.90	16.22	89.98	10.02
R_2	56.90	4.95	1.93	96.14	13.85	90.92	9.08
V_1	56.83	5.86	2.84	94.32	12.48	84.45	15.55
V_2	57.08	7.98	4.96	90.08	14.25	89.76	10.24
G	56.94	5.85	2.83	94.34	11.18	79.41	20.59
Subject No. 3 ; 24 yrs. ; wt., 50.4 kg.							
B	6.59	2.59	..	..	8.02	41.58	58.42
C_1	56.69	6.28	3.69	92.62	24.75	75.85	24.15
C_2	56.60	6.45	3.86	92.28	24.06	81.08	18.92
R_1	56.73	6.64	4.05	91.90	26.43	76.15	23.85
R_2	56.65	6.78	4.19	91.62	25.83	72.18	27.82
V_1	56.70	7.17	4.58	90.84	22.44	79.02	20.98
V_2	56.76	8.89	6.30	87.40	23.15	78.65	21.35
G	56.79	7.52	4.93	90.14	20.20	88.74	11.26
Subject No. 4 ; 25 yrs. ; wt., 48.0 kg.							
B	7.19	3.08	..	..	6.90	54.82	45.18
C_1	57.22	5.48	2.40	95.20	14.28	92.15	7.85
C_2	57.28	5.31	2.23	95.54	12.40	86.55	13.45
R_1	57.32	4.81	1.73	96.54	18.01	82.94	17.06
V_1	57.28	6.51	3.43	93.14	18.29	90.15	9.85
V_2	57.36	8.42	5.34	89.32	17.08	73.29	26.71
G	57.32	6.13	3.05	93.90	17.24	77.51	22.49

Diet	Av. daily fat intake	Av. daily fat excretion.	Av. daily fat excretion due to added fat	% Absorption of added fat	% Absorption in dried faeces	% faecal fat	
						split fat	unsplit fat
	g.	g.	g.				
			Subject No. 5 ; 22 yrs. ; wt., 56.8 kg.				
B	7.02	2.98	..	..	7.56	51.99	48.01
C_1	57.18	5.18	2.20	95.60	10.85	86.29	13.71
C_2	57.12	5.24	2.26	95.48	9.22	90.25	9.75
R_1	57.22	4.43	1.45	97.10	12.83	87.44	12.56
R_2	57.22	4.56	1.58	96.84	10.92	85.54	14.46
V_1	57.14	6.01	3.03	93.94	12.05	93.85	6.15
V_2	57.20	6.47	3.49	93.02	13.53	81.73	18.27
G	57.18	5.45	2.47	95.06	16.81	74.80	25.20
			Subject No. 6 ; 24 yrs. ; wt., 45.0 kg.				
B	7.19	3.14	..	..	8.01	55.51	44.49
C_1	57.19	5.73	2.59	94.82	12.44	88.08	11.92
C_2	57.22	5.92	2.78	94.44	10.99	94.28	5.72
R_1	57.28	5.49	2.35	95.30	12.09	82.62	17.38
R_2	57.22	5.67	2.53	94.94	10.17	82.48	17.52
V_1	57.25	6.96	3.82	92.36	9.98	85.45	14.55
V_2	57.26	9.03	5.89	88.22	16.23	87.11	12.89
G	57.29	6.72	3.58	92.84	12.25	91.93	8.07
			Subject No. 7 ; 23 yrs. ; wt., 54.9 kg.				
B	7.36	3.22	..	..	6.80	48.15	51.85
C_1	57.34	5.12	1.90	96.20	10.85	79.92	20.08
C_2	57.36	4.94	1.72	96.56	8.72	82.18	17.82
R_1	57.42	4.21	0.99	98.02	8.99	88.25	11.75
R_2	57.42	4.41	1.19	97.62	11.94	83.43	16.57
V_1	57.36	5.60	2.38	95.24	12.05	92.02	7.98
V_2	57.44	7.59	4.37	91.26	17.85	75.29	24.71
G	57.48	4.90	1.68	96.64	13.08	87.25	12.75
			Subect No. 8 ; 35 yrs. ; wt., 51.3 kg.				
B	6.92	2.80	..	..	9.12	32.90	67.10
C_1	56.50	6.25	3.45	93.10	16.19	70.22	29.78
C_2	57.03	6.03	3.23	93.54	20.25	75.20	24.80
R_1	57.03	5.85	3.05	93.90	18.98	78.33	21.67
R_2	57.12	5.62	2.82	94.36	17.40	69.90	30.10
V_1	57.12	7.03	4.23	91.54	24.09	69.28	30.72
V_2	57.10	9.69	6.89	86.22	16.72	84.20	15.80
G	57.10	6.79	3.99	92.02	22.43	73.25	26.75
			Subject No. 9 ; 24 yrs. ; wt., 45.0 kg.				
B	6.42	2.41	..	..	8.61	36.22	63.78
C_1	56.50	6.74	4.33	91.34	25.22	72.10	27.90
C_2	56.48	6.83	4.42	91.16	20.15	74.20	25.80
R_1	56.50	6.21	3.80	92.40	24.03	68.99	31.01
R_2	56.60	6.34	3.93	92.14	22.76	78.20	21.80
V_1	56.42	7.47	5.06	89.88	28.40	76.62	23.38
G	56.55	6.81	4.40	91.20	19.21	72.17	27.83
			Subject No. 10 ; 22 yrs. ; wt., 56.7 kg.				
B	7.22	3.53	..	..	6.25	29.57	70.43
C_1	57.33	4.87	1.34	97.32	8.98	82.08	17.92
C_2	57.33	5.08	1.55	96.90	11.18	70.55	29.45
R_1	57.40	4.12	0.59	98.82	12.43	68.02	31.98
R_2	57.40	3.96	0.43	99.14	7.99	72.88	27.12
V_1	57.38	5.73	2.20	95.60	10.92	71.29	28.71
V_2	57.35	7.48	3.95	92.10	13.83	88.05	11.95
G	57.38	5.67	2.14	95.72	16.05	90.30	9.70

Diet	Av. daily fat intake	Av. daily fat excretion.	Av. daily fat excretion due to added fat	% Absorption of added fat	% Absorption in dried faeces	% faecal fat: split fat	% faecal fat: unsplit fat
	g.	g.	g.				
Subject No. 11 ; 22 yrs. ; wt., 49.5 kg.							
B	7.22	3.48	..	..	4.20	52.81	47.19
C_1	57.30	5.08	1.60	96.80	10.12	78.82	21.18
C_2	57,30	5.35	1.87	96.26	7.38	72.80	27.20
R_1	57.38	4.61	1.13	97.74	8.05	78.22	21.78
R_2	57.38	4.43	0.95	98.10	9.65	86.88	13.12
G	57.29	6.90	3.42	93.16	11.92	91.15	8.85
Subject No. 12 ; 25 yrs. ; wt., 55.8 kg.							
B	7.36	3.47	..	..	4.50	56.12	43.88
C_1	57.36	4.96	1.49	97.02	6.85	90.94	9.96
C_2	57.36	4.81	1.34	97.32	7.28	80.96	19.04
R_1	57.40	3.87	0.40	99.20	7.08	85.28	14.72
R_2	57.40	4.00	0.53	98.94	8.17	78.99	21.01
V_1	57.44	5.76	2.29	95.42	6.69	88.05	11.95
V_2	57.45	7.09	3.62	92.76	8.29	70.04	29.96
G	57.41	5.40	1.93	96.14	7.14	89.79	10.21

Table 3: Range of absorption of raw and refined groundnut oils, vanasapatis and ghee

Fat	% absorption of added fat: lowest	highest	Av.	No. of observations
Raw G. N. oil	91.16	97.32	94.97	20
Refined G. N. oil	91.62	99.20	96.03	21
Vanaspati, m.p., 37°C. ..	89.88	95.60	93.23	10
Vanaspati, m.p., 41°C. ..	86.22	93.02	90.04	9
Ghee	90.14	96.64	93.74	11

Table 4: Range of hydrolysis of faecal fats

Fat	% hydrolysis of faecal fat: lowest	highest	Av.	No. of observations
No fat added	29.57	56.12	46.24	11
Raw G. N. oil	70.22	94.28	80.14	24
Refined G. N. oil	68.02	90.92	80.25	23
Vanaspati, m.p., 37 C. ..	69.28	93.85	81.85	11
Vanaspati, m.p., 41 °C. ..	70.04	89.76	80.90	9
Ghee	72.17	91.93	83.30	11

Table 5: Composition of plasma lipoids

Per 100 cc. of blood plasma

Subject No.	Diet	cholesterol mg.	fatty acids mg.	Iod. no. plasma fatty acids
1	C_1	155	326	156
	R_2	164	344	149
	V_1	158	326	128
2	C_2	198	386	160

Subject	Diet	Per 100 cc. blood plasma		Iod. no. plasma fatty acids
		cholesterol mg.	fatty acids mg.	
2A	B	152	269	115
	R_2	178	356	172
	V_1	184	352	146
	V_2	176	343	138
3	B	138	241	108
	C_2	146	299	150
	V_1	150	312	128
	V_2	157	309	133
4	B	154	294	119
	C_1	173	328	164
	C_2	177	369	160
	V_1	173	363	150
	V_2	171	340	140
	G	178	336	139
5	B	162	300	112
	C_1	187	380	160
	C_2	193	394	166
	R_2	198	401	160
	V_1	195	382	142
	V_2	190	391	146
6	B	147	265	112
	C_2	177	342	164
	R_2	186	372	164
	V_1	169	328	138
	V_2	164	323	140
	G	171	336	135
7	B	133	248	104
	C_1	158	309	156
	R_2	156	328	149
	V_1	163	346	133
	V_2	163	317	130
	G	169	349	138
8	C_1	172	328	150
	C_2	181	362	154
	R_2	188	380	158
	V_2	195	398	145
	G	194	389	141
9	B	126	228	106
	C_1	148	281	152
	R_2	158	296	154
	V_1	143	278	130
	G	146	301	127
10	B	141	262	115
	C_1	168	342	166
	C_2	163	318	168
	V_1	160	309	148
	V	154	328	142
	G	160	313	144
11	B	158	290	117
	C_1	191	394	162
	C_2	184	377	162
	R_2	194	396	172
	G	182	375	144

Subject No.	Diet	Per 100 cc. of blood plasma		Iod. no. plasma fatty acids
		cholesterol mg.	fatty acids mg.	
12	B	139	245	121
	C_1	155	320	165
	C_2	153	306	168
	R_2	167	349	168
	V_1	173	339	150
	G	160	344	144

Table 6: Relationship between dietary fats and plasma lipoids

Fat	Iod. no. of fat	no. of observations	Iod. no. of plasma fatty acids		
			lowest	highest	av.
No fat added ..	..	10	104	121	112.9
Raw G. N. oil ..	96	18	150	168	160.6
Refined G. N. oil ..	93	9	149	172	160.6
Vanaspati, m.p., 37°C.	66	10	128	150	139.3
Vanaspati, m.p., 41°C.	62	8	130	146	140.2
Ghee	41	8	127	144	139.0

B—Influence of ingested fats on calcium, phosphorus and protein metabolism

The addition of moderate amounts of fats to a fat-free diet has been reported to have a beneficial influence on calcium and phosphorus utilisation[33-37], although the retention of calcium tends to be lowered with higher amounts of dietary fat[37,39]. The anti-rachitic effect of vitamin D-free fats has been found to be partly due to the influence of fat on calcium and phosphorus absorption.[35,36,40-48] According to several investigators[44,49-52], vitamin D increases, or is a pre-requisite to, the absorption of calcium and phosphorus, whereas according to others[53-57], vitamin D does not assist in the absorption of calcium and phosphorus from the intestines.

Considerable emphasis has been laid on the Ca P ratio as a factor affecting the calcium and phosphorus metabolism[48, 58, 59]. A low protein diet may lead to negative calcium and phosphorus balance, and the absorption of calcium is increased when extra proteins or amino acids are administered[60-62]. Vitamin C also influences calcium metabolism. Givens[63,64] has indicated that all calcium soaps are not absorbed equally[34,65]. Oleo oil slightly interferes with calcium utilisation in rats.[39] Basu and Nath[10] have reported that the use of coconut oil as the main dietary fat may affect calcium utilisation in human beings.

Very little work has been done on the influence of dietary fat on protein metabolism. Forbes *et al.*[69] reported that in young or adult rats the digestibility of protein and the retention of food nitrogen are in the order of the increasing fat content of the diet.

Recently, Kehar and Chanda[67] reported that the biological value of protein as determined in experiments with rats receiving cow ghee is definitely higher than that determined in experiments with rats receiving vegetable oil or vanaspati ; also the digestibility coefficient of protein is

decreased by 26 per cent and the biological value by 38 per cent on a fat-free diet as compared to a diet containing ghee. Boutwell *et al.*[68] have indicated that the requirements of B vitamins for the successful utilisation of dietary protein are enhanced when the diet contains corn oil. Pittman and Kunerth[69] have concluded that medium-protein diet improves appreciably the utilisation of nitrogen, calcium and phosphorus in human subjects compared with low-protein diet.

Sherman's standards for the adequate requirements of calcium and phosphorus for an adult human are 0.63 g. calcium and 0.98 g. phosphorus per day. He recommends a standard allowance of 1.0 g. of protein per kilo of body weight per day[70]. The diet used in the present investigation supplied on the average 0.5) g. calcium, 1.23 g. phosphorus and 60.43 g. protein per head per day and was, therefore, adequate in protein and phosphorus contents but was slightly deficient in calcium. The Ca/P ratio of the diet supplied was on the average 1/2.54.

In the present section, the comparative effects of raw and refined groundnut oils, vanaspatis, m.p., 37°C. and 41°C., and ghee on calcium, phosphorus and protein metabolism on the same subjects have been studied.

Analytical Methods—Phosphorus in foods, faeces and urine was determined by the method of Fiske and Subbarow[71] as modified by King[72]. Calcium in urine was determined by the method of Shohl and Pedley[73]. Foods and faeces were dry ashed by McCrudden's method[74] and calcium was determined in the ash solution.

The results relating to calcium, phosphorus and protein metabolism on practically fat-free as well as fat-supplemented diets for individual subjects are given in Tables 7-9.

Table 7: Effect of raw and refined groundnut oils, vanaspatis and ghee on calcium metabolism

Subject No.	Diet	Calcium				Balance
		Av. daily intake	Av. daily urinary output	Av. daily faecal output	Av. daily total output	
		g.	g.	g.	g.	g.
1	C_1	0.494	0.101	0.386	0.487	0.007
	C_2	0.494	0.128	0.263	0.391	0.103
	R_1	0.496	0.032	0.435	0.517	−0.021
	R_2	0.496	0.142	0.311	0.453	0.043
	V_1	0.496	0.039	0.408	0.507	−0.011
2	C_1	0.506	0.138	0.392	0.531	−0.025
	C_2	0.506	0.151	0.338	0.489	0.017
2A	B	0.504	0.120	0.464	0.584	−0.080
	R_1	0.506	0.127	0.244	0.371	0.135
	R_2	0.506	0.110	0.298	0.408	0.098
	V_1	0.504	0.086	0.314	0.400	0.104
	V_2	0.508	0.190	0.309	0,499	0.009
	G	0.508	0.207	0.239	0.446	0.062

Subject No.	Diet	Calcium				Balance
		Av. daily intake	Av. daily urinary output	Av. daily faecal output	Av. daily total output	
		g.	g.	g.	g.	g.
3	B	0.498	0.155	0.427	0.582	−0.084
	C_1	0.502	0.128	0.283	0.411	0.091
	C_2	0.500	0.099	0.283	0.382	0.118
	R_1	0.504	0.147	0.253	0.400	0.104
	R_2	0.502	0.103	0.272	0.375	0.127
	V_1	0.504	0.118	0.274	0.392	0.112
	V_2	0.502	0.079	0.287	0.366	0.136
	G	0.504	0.182	0.199	0.381	0.123
4	B	0.513	0.139	0.436	0.575	−0.062
	C_1	0.515	0.127	0.300	0.427	0.088
	C_2	0.517	0.101	0.236	0.337	0.180
	R_1	0.519	0.089	0.277	0.366	0.153
	V_1	0.517	0.109	0.306	0.415	0.102
	V_2	0.519	0.259	0.273	0.532	−0.013
	G	0.517	0.134	0.302	0.436	0.081
5	B	0.509	0.145	0.450	0.595	−0.033
	C_1	0.513	0.142	0.272	0.414	0.099
	C_2	0.511	0.090	0.378	0.468	0.043
	R_1	0.515	0.157	0.249	0.403	0.109
	R_2	0.515	0.139	0.380	0.519	−0.004
	V_1	0.511	0.117	0.263	0.380	0.131
	V_2	0.514	0.114	0.281	0.395	0.119
	G	0.514	0.078	0.297	0.375	0.139
6	B	0.513	0.138	0.448	0.586	−0.073
	C_1	0.511	0.130	0.238	0.368	0.143
	C_2	0.514	0.109	0.234	0.343	0.171
	R_1	0.517	0.123	0.313	0.436	0.081
	R_2	0.515	0.076	0.334	0.410	0.105
	V_1	0.517	0.139	0.245	0.384	0.133
	V_2	0.517	0.076	0.390	0.466	0.051
	G	0.518	0.218	0.302	0.520	−0.002
7	B	0.522	0.119	0.431	0.550	−0.028
	C_1	0.520	0.105	0.223	0.328	0.192
	C_2	0.522	0.101	0.273	0.374	0.148
	R_1	0.525	0.112	0.286	0.398	0.127
	R_2	0.525	0.127	0.400	0.527	−0.002
	V_1	0.522	0.114	0.300	0.414	0.108
	V_2	0.525	0.160	0.292	0.452	0.073
	G	0.527	0.185	0.226	0.411	0.116
8	B	0.506	0.163	0.409	0.572	−0.066
	C_1	0.504	0.162	0.332	0.495	0.009
	C_2	0.508	0.112	0.286	0.398	0.110
	R_1	0.508	0.130	0.249	0.379	0.129
	R_2	0.510	0.148	0.270	0.418	0.092
	V_1	0.512	0.157	0.276	0.433	0.079
	V_2	0.508	0.148	0.363	0.511	−0.003
	G	0.510	0.140	0.288	0.428	0.082
9	B	0.494	0.109	0.478	0.587	−0.093
	C_1	0.497	0.118	0.397	0.515	−0.018
	C_2	0.495	0.112	0.333	0.445	0.050
	R_1	0.497	0.094	0.321	0.415	0.082
	R_2	0.497	0.038	0.303	0.394	0.103
	V_1	0.492	0.101	0.401	0.502	−0.010
	G	0.498	0.180	0.301	0.481	0.017
10	B	0.516	0.170	0.385	0.555	−0.039
	C_1	0.519	0.129	0.398	0.527	−0.008

Subject No.	Diet	Calcium				Balance
		Av. daily intake	Av. daily urinary output	Av. daily faecal output	Av. daily total output	
		g.	g.	g.	g.	g.
	C_2	0.519	0.158	0.312	0.470	0.049
	R_1	0.522	0.167	0.294	0.461	0.031
	R_2	0.522	0.113	0.312	0.425	0.097
	V_1	0.520	0.144	0.343	0.487	0.033
	V_2	0.519	0.057	0.350	0.407	0.112
	G	0.521	0.115	0.281	0.396	0.125
11	B	0.516	0.143	0.435	0.578	−0.062
	C_1	0.519	0.118	0.282	0.400	0.119
	C_2	0.519	0.138	0.290	0.428	0.091
	R_1	0.521	0.140	0.229	0.369	0.152
	R_2	0.521	0.129	0.313	0.442	0.079
	G	0.519	0.182	0.196	0.378	0.141
12	B	0.522	0.151	0.415	0.566	−0.044
	C_1	0.522	0.151	0.377	0.523	−0.003
	C_2	0.522	0.140	0.370	0.510	0.012
	R_1	0.524	0.148	0.236	0.384	0.140
	R_2	0.524	0.118	0.278	0.395	0.129
	V_1	0.526	0.139	0.214	0.353	0.173
	V_2	0.526	0.038	0.285	0.373	0.153
	G	0.525	0.116	0.250	0.366	0.159

Table 8: Effect of raw and refined groundnut oils, vanaspatis and ghee on phosphorus metabolism

Subject No.	Diet	Phosphorus				Balance
		Av. daily intake	Av. daily urinary output	Av. daily faecal output	Av. daily total output	
		g.	g.	g.	g.	g.
1	C_1	1.150	0.648	0.410	1.058	0.092
	C_2	1.150	0.727	0.318	1.045	0.105
	R_1	1.179	0.677	0.375	1.052	0.127
	R_2	1.179	0.732	0.295	1.027	0.152
	V_1	1.175	0.777	0.260	1.037	0.138
2	C_1	1.309	0.717	0.394	1.111	0.198
	C_2	1.309	0.809	0.290	1.099	0.210
2A	B	1.282	0.685	0.501	1.186	0.096
	R1	1.308	0.704	0.488	1.192	0.116
	R2	1.308	0.730	0.430	1.160	0.148
	V_1	1.278	0.674	0.484	1.154	0.124
	V_2	1.319	0.592	0.626	1.218	0.101
	G	1.314	0.581	0.567	1.148	0.166
3	B	1.178	0.681	0.421	1.102	0.076
	C_1	1.207	0.638	0.368	1.006	0.201
	C_2	1.202	0.665	0.308	0.973	0.229
	R_1	1.233	0.682	0.404	1.086	0.147
	R_2	1.205	0.624	0.392	1.016	0.189
	V_1	1.233	0.707	0.303	1.015	0.218
	V_2	1.218	0.701	0.312	1.013	0.205
	G	1.229	0.613	0.419	1.032	0.197
4	B	1.368	0.864	0.391	1.255	0.113
	C_1	1.391	0.786	0.320	1.106	0.285

Subject No.	Diet	Phosphorus				Balance
		Av. daily intake	Av. daily urinary output	Av. daily faecal output	Av. daily total output	
		g.	g.	g.	g.	g.
	C_2	1.406	0.815	0.399	1.214	0.192
	R_1	1.423	0.812	0.297	1.109	0.314
	V_1	1.401	0.823	0.271	1.104	0.297
	V_2	1.417	0.668	0.471	1.139	0.278
	G	1.412	0.705	0.386	1.091	0.321
5	B	1.338	0.841	0.409	1.250	0.088
	C_1	1.367	0.855	0.416	1.271	0.096
	C_2	1.352	0.781	0.401	1.182	0.170
	R_1	1.394	0.789	0.390	1.179	0.215
	R_2	1.394	0.845	0.350	1.195	0.199
	V_1	1.359	0.822	0.334	1.156	0.203
	V_2	1.377	0.795	0.430	1.225	0.152
	G	1.370	0.713	0.488	1.201	0.169
6		1.368	0.830	0.440	1.270	0.098
	C_1	1.368	0.661	0.397	1.058	0.310
	C_2	1.388	0.796	0.410	1.206	0.182
	R_1	1.398	0.861	0.318	1.179	0.219
	R_2	1.394	0.730	0.388	1.118	0.276
	V_1	1.394	0.810	0.295	1.105	0.289
	V_2	1.403	0.696	0.414	1.110	0.293
	G	1.411	0.748	0.322	1.070	0.341
7	B	1.428	0.882	0.418	1.300	0.128
	C_1	1.422	0.888	0.440	1.328	0.094
	C_2	1.434	0.895	0.400	1.295	0.139
	R_1	1.455	0.875	0.388	1.263	0.192
	R_2	1.458	0.877	0.371	1.248	0.210
	V_1	1.436	0.861	0.350	1.211	0.225
	V_2	1.464	0.697	0.634	1.331	0.133
	G	1.471	0.571	0.750	1.321	0.150
8	B	1.309	0.838	0.409	1.247	0.062
	C_1	1.309	0.880	0.319	1.199	0.110
	C_2	1.335	0.863	0.334	1.197	0.138
	R_1	1.337	0.788	0.380	1.168	0.169
	R_2	1.364	0.864	0.361	1.225	0.139
	V_1	1.364	0.796	0.390	1.186	0.178
	V_2	1.343	0.840	0.381	1.221	0.122
	G	1.350	0.886	0.354	1.240	0.110
9	B	1.150	0.711	0.385	1.096	0.054
	C_1	1.177	0.633	0.362	0.995	0.182
	C_2	1.154	0.646	0.382	1.028	0.126
	R_1	1.179	0.720	0.300	1.020	0.150
	R_2	1.177	0.717	0.290	1.007	0.170
	V_1	1.152	0.649	0.312	0.961	0.191
	G	1.179	0.710	0.268	0.978	0.201
10	B	1.399	0.754	0.510	1.264	0.135
	C_1	1.427	0.795	0.493	1.288	0.139
	C_2	1.427	0.796	0.481	1.277	0.150
	R_1	1.454	0.792	0.470	1.262	0.192
	R_2	1.454	0.827	0.421	1.248	0.203
	V_1	1.449	0.801	0.405	1.266	0.183
	V_2	1.441	0.805	0.438	1.243	0.198
	G	1.456	0.892	0.283	1.175	0.281
11	B	1.399	0.809	0.439	1.248	0.151
	C_1	1.427	0.992	0.430	1.222	0.205
	C_2	1.427	0.757	0.409	1.166	0.261

Subject No.	Diet	Phosphorus				Balance
		Av. daily intake	Av. daily urinary output	Av. daily faecal output	Av. daily total output	
		g.	g.	g.	g.	g.
	R_1	1.550	0.836	0.397	1.233	0.317
	R_2	1.550	0.884	0.370	1.254	0.296
	G	1.438	0.715	0.422	1.137	0,301
12	B	1.428	0.717	0.548	1.265	0.163
	C_1	1.431	0.723	0.550	1.273	0.158
	C_2	1.431	0.713	0.538	1.251	0.180
	R_1	1.455	0.734	0.530	1.264	0.191
	R_2	1.455	0.745	0.491	1.236	0.219
	V_1	1.458	0.735	0.515	1.250	0.208
	V_2	1.479	0.645	0.620	1.265	0.214
	G	1.463	0.713	0.501	1.214	0.249

Table 9: Effect of raw and refined groundnut oils, vanaspatis and ghee on protein metabolism

Subject No.	Diet	Av. daily dietary N intake	Av. daily urinary N output	Av. daily faecal N output	Av. daily total N output	Balance
		g.	g.	g.	g.	g.
1	C_1	8.339	4.864	1.750	6.614	1.725
	C_2	8.334	4.815	1.618	6.433	1.901
	R_1	8.602	5.048	1.214	6.262	2.340
	R_2	8.602	4.632	1.785	6.417	2.185
	V_1	8.571	4.581	1.501	6.082	2.489
3	C_1	9.942	5.592	1.400	6.992	2.950
	C_2	9.949	6.045	1.109	7.154	2.795
2A	B	9.668	5.407	2.110	7.517	2.151
	R_1	9.937	5.451	1.906	7.357	2.580
	R_2	9.943	5.640	1.428	7.068	2.875
	V_1	9.673	5.383	1.390	6.773	2.900
	V_2	9.953	5.112	1.825	6.937	3.016
	G	9.941	5.370	1.679	7.049	2.892
4	B	8.676	5.285	1.981	7.266	1.410
	C_1	8.950	5.358	1.410	6.768	2.182
	C_2	8.904	5.294	1.381	6.675	2.229
	R_1	9.224	5.129	1.996	7.125	2.099
	R_2	8.926	5.382	1.204	6.586	2.340
	V_1	9.212	5.165	1.752	6.917	2.295
	V_2	9.203	5.335	1.795	7.130	2.073
	G	9.218	5.302	1.720	7.022	2.196
5	B	10.512	5.730	1.878	7.608	2.904
	C_1	10.765	5.610	1.904	7.514	3.251
	C_2	10.780	5.280	2.110	7.390	3.390
	R_1	10.792	5.317	2.201	7.518	3.274
	V_1	10.772	5.453	2.018	7.471	3.301
	V_2	10.910	5.302	2.340	7.642	3.268
	G	10.861	5.604	2.058	7.662	3.199
5	B	10.210	6.160	1.720	7.880	2.330
	C_1	10.489	6.338	1.092	7.430	3.059
	C_2	10.476	5.765	1.190	6.955	3.521
	R_1	10.756	6.078	1.759	7.837	2.919
	R_2	10.756	5.904	1.582	7.486	3.270
	V_1	10.480	5.257	1.813	7.070	3.410
	V_2	10.742	5.722	1.912	7.634	3.108
	G	10.709	5.817	1.899	7.716	2.993

Subject No.	Diet	Av. daily dietary N intake g.	Av. daily urinary N output g.	Av. daily faecal N output g.	Av. daily total N output g.	Balance g.
6	B	10.512	6.343	1.648	7.991	2.521
	C_1	10.515	6.156	1.489	7.645	2.870
	C_2	10.760	5.722	1.920	7.642	3.118
	R_1	10.789	6.082	1.751	7.833	2.956
	R_2	10.766	5.457	2.019	7.476	3.290
	V_1	10.768	5.448	2.110	7.558	3.210
	V_2	10.759	6.018	1.990	8.008	2.751
	G	10.818	5.707	2.018	7.725	3.093
7	B	11.042	6.160	1.902	8.062	2.980
	C_1	11.046	5.499	2.118	7.617	3.429
	C_2	11.052	5.794	1.750	7.544	3.508
	R_1	11.292	5.988	1.680	7.668	3.624
	R_2	11.292	5.878	2.004	7.882	3.410
	V_1	11.058	5.874	1.583	7.457	3.601
	V_2	11.301	5.874	2.115	7.989	3.312
	G	11.317	5.763	2.157	7.920	3.397
8	B	9.942	6.724	1.480	8.204	1.738
	C_1	9.968	6.338	1.410	7.748	2.220
	C_2	10.214	5.481	2.118	7.599	2.615
	R_1	10.219	5.169	2.548	7.717	2.502
	R_2	10.480	6.259	1.900	8.159	2.321
	V_1	10.495	6.201	1.753	7.954	2.541
	V_2	10.487	6.019	2.168	8.187	2.300
	G	10.493	5.969	2.283	8.252	2.241
9	B	8.339	5.891	1.208	7.099	1.240
	C_1	8.602	5.557	1.005	6.562	2.040
	C_2	8.588	4.664	1.823	6.487	2.101
	R_1	8.609	5.029	1.610	6.639	1.970
	R_2	8.609	5.508	1.503	7.011	1.598
	V_1	8.348	5.139	1.405	6.544	1.804
	G	8.612	4.730	1.920	6.650	1.962
10	B	10.761	6.047	2.149	8.196	2.565
	C_1	11.002	5.002	2.710	7.712	3.290
	C_2	11.039	5.720	1.918	7.638	3.401
	R_1	11.282	5.414	2.588	8.002	3.280
	R_2	11.279	5.169	2.916	8.085	3.194
	V_1	11.264	5.891	1.850	7.741	3.523
	V_2	11.040	5.753	2.283	8.036	3.004
	G	11.052	5.769	2.158	7.927	3.125
11	B	10.761	6.352	1.754	8.106	2.655
	C_1	10.962	5.752	1.920	7.672	3.290
	C_2	10.951	6.151	1.699	7.850	3.101
	R_1	11.278	5.697	2.388	8.085	3.193
	R_2	11.264	5.831	2.103	7.934	3.330
	G	11.082	6.037	1.890	7.927	3.155
12	B	11.042	5.922	1.910	7.832	3.210
	C_1	11.052	6.177	1.431	7.608	3.444
	C_2	11.052	5.978	1.373	7.351	3.701
	R_1	11.288	5.493	2.103	7.596	3.692
	R_2	11.288	5.430	2.018	7.448	3.840
	V_1	11.301	5.315	2.228	7.543	3.758
	V_2	11.319	5.580	2.188	7.768	3.551
	G	11.297	5.707	1.978	7.685	3.612

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SECTION II

Experiments conducted at the Nutrition Research Laboratories, Coonoor.*

The experiments were carried out with six human volunteers. The diet given to each was the poor rice diet supplemented by 68 g. of one or the other of the following fats per head (i.e., *c.* 10 per cent of the total diet).

1. Raw groundnut oil
2. Refined groundnut oil
3. Hyderogenated groundnut oil (vanaspati, m.p., 37°C.)
4. Hydrogenated groundnut oil (vanaspati, m.p., 41°C.)
5. Ghee

EXPERIMENTAL PROCEDURE

Six healthy adult males volunteered for the present study. Adults were considered suitable subjects for experiments since in them the storage of nitrogen, calcium and phosphorus as a factor in growth has ceased to operate. Detailed information about the subjects is given in Table 1. All of them were laboratory employees and they were engaged in routine activities during the experimental period. Two subjects were taken at a time for experiment.

Table 1: Information on experimental subjects

Subject No.	Occupation	Age years	Weight initial lb.	Weight final lb.	Height ft.	Height in.	Days under observation
1	Lab. assistant ..	28	100	101	5	2	50
2	Lab. servant	29	127	129	5	4	49
3	do.	29	113	117	5	8	56
4	do.	27	132	136	5	7	49
5	do.	36	113	117.5	5	5	52
6	do.	28	129	132	5	5	52

The total period of observation for each subject was divided into five periods of 10 days each. Before a subject was taken up for experiment, he was put on a trial diet to find out the quantities of rice which were necessary to satisfy him. Taking this quantity of rice as the starting point, other constituents of the diet were varied in order to keep their proportion in the agreed diet fairly constant. The quantity of fat fed per day was, however, constant for all subjects at the level of 68 g. per subject per day.

*The work described in this section was carried out by Shri P. G. Tulpule under the supervision of Dr. V. N. Patwardhan.

The first five days of each test period were used for the purpose of allowing the subject to come to a constant metabolic condition with regard to the particular diet. The second five days period was used for the collection of excreta. These were collected on two consecutive days, followed by a gap of one day, and a second collection for the next consecutive two-day period.

The composition of the poor rice diet used in the experiments was as follows (as supplemented by oils) :

	oz.	g.	%
Polished rice	18.6	530.0	76.7
Pulse (tur dal)	0.7	20.0	2.1
Non-leafy vegetables	2.0	57.0	8.2
Leafy vegetables	0.5	15.0	2.1
Whole milk powder (Klim)	0.2	5.7	0.9
Oil (supplement)	2.4	68.4	10.0

Equal parts of potatoes, carrots and cauliflower formed the non-leafy vegetables; cabbage was served as the leafy vegetable. The quntities of diets given to individual subjects are given in Table 2.

Table 2: Food intake by subjects

	Subject No. 1 oz.	Subject No. 2 oz.	Subjects Nos. 3,4,5 & 6 oz.
Rice	18.0	22.0	26.0
Pulse (tur dhal)	0.7	0.85	1.0
Non-leafy vegetables & potatoes (equal)	2.0	2.5	3.0
Leafy vegetables (cabbage) ..	0.5	0.65	0.75
Whole milk powder (Klim) ..	0.2	0.25	0.3
Oil or fat	2.4	2.4	2.4
Composition of cooked diet without added fat			
Nitrogen (g.)	7.0207	8.5808	10.1410
Phosphorus (g.)	0.6298	0.7698	0.9097
Calcium (g.)	0.4527	0.5533	0.6539

The order of feeding supplementary fat was the same for all subjects.

Cooking and feeding—The foodstuffs were weighed and the diet cooked in the laboratory kitchen three times a day under supervision; the method of cooking was the same throughout the experiment. The major portion of the fat supplement was consumed at the time of breakfast in the form of a typical South Indian dish, i.e., 'Dosai' prepared from rice. The vegetables and pulse were consumed in the form of curry during lunch and dinner. The remaining portion of the fat supplement was consumed at these two meals. The whole milk powder was diluted ten times with water and used with tea in the morning. Feeding of subjects was closely supervised.

Collection and analysis of urine and faeces—1. Urine: Twenty-four hour samples of urine were collected for quantitative estimation of calcium, phosphorus, nitrogen and creatinine. The creatinine excreted by a nor-

mal adult is nearly constant from day to day and is hardly influenced by the diet unless the latter contains much preformed creatinine. The creatinine estimation, therefore, served as a check over the collection of urine as a 24-hour specimen.

Faeces—The faeces excreted by the subject during 24-hour periods were collected in flat basins and dried on a boiling water bath, the final drying being carried out in a steam oven. The dried faeces of two days from the same subject were mixed, weighed, powdered and used for the quantitative estimation of calcium, phosphorus, total nitrogen and ether extract. The results are given in Tables 3 and 4.

Table 3: Excretion of fat in faeces per 2-day period

Fat	Faeces dry wt. g.	Faecal fat g.	Faecal fat × 100 / Faeces (dry wt.)	Fat excreted as % of intake
Subject No. 1				
Raw G. N. oil	57.5	6.77	11.78	4.98
	51.0	5.51	10.81	4.05
Refined G. N. oil	70.4	7.14	10.14	5.25
	68.4	7.40	10.81	5.44
Vanaspati, m. p., 37°C. ..	49.2	5.69	11.56	4.11
	70.6	6.99	9.90	5.14
Vanaspati, m. p., 41°C. ..	74.8	17.53	23.44	12.89
	87.3	21.62	24.76	15.82
Ghee	53.1	5.76	10.86	4.24
	60.9	6.61	10.88	4.86
Subject No. 2				
Raw G. N. oil	63.5	8.22	12.96	6.05
	41.0	6.40	15.62	4.70
Refined G. N. oil ..	46.7	7.10	15.21	5.22
	54.0	7.78	14.40	5.72
Vanaspati, m. p., 37°C. ..	98.1	9.26	9.44	6.80
	64.1	8.74	13.64	6.42
Vanaspati, m. p., 41°C. ..	49.3	8.78	17.82	6.45
	70.7	12.69	17.96	9.33
Ghee	77.2	8.16	10.57	6.00
	65.4	8.09	12.38	5.75
Subject No. 3				
Raw G. N. oil	66.2	9.71	14.51	7.14
	60.6	8.43	14.05	6.20
Refined G. N. oil ..	65.8	9.33	14.19	6.86
	80.1	11.03	13.77	8.11
Vanaspati, m. p., 37°C. ..	75.4	11.50	15.12	8.46
	63.1	10.22	16.20	7.52
Vanaspati, m. p., 41°C. ..	74.4	15.75	21.17	11.58
	56.0	11.26	20.12	8.28
Ghee	51.1	8.35	16.35	6.64
	69.4	10.00	14.42	7.35

Fat	Faeces dry wt. g.	Faecal fat g.	Faecal fat × 100 / Faeces (dry wt.)	Fat excreted as % of intake
Subject No. 4				
Raw G. N. oil	51.3	5.76	11.23	4.23
	52.6	6.47	12.30	4.76
Refined G. N. oil ..	59.9	6.71	11.21	4.93
	76.7	8.32	10.85	6.11
Vanaspati, m. p., 37°C. ..	59.6	7.17	12.04	5.27
	49.7	7.86	15.83	5.78
Vanaspati, m. p., 41°C. ..	68.2	12.57	18.44	9.24
	67.7	14.33	21.17	10.53
Ghee	49.1	7.09	14.44	5.21
	43.3	6.52	15.06	4.79
Subject No. 5				
Raw G. N. oil	78.4	8.02	10.24	5.90
	66.9	7.56	11.30	5.56
Refined G. N. oil	77.8	7.78	10.01	5.72
	81.3	7.97	9.81	5.86
Vanaspati, m. p., 37°C. ..	65.3	6.78	10.39	4.98
	80.0	7.30	9.13	5.37
Vanaspati, m. p., 41°C. ..	64.6	8.63	13.36	6.34
	83.6	9.23	11.05	6.79
Ghee	67.4	7.23	10.73	5.31
	51.6	6.48	12.55	4.76
Subject No. 6				
Raw G. N. oil	77.0	11.91	15.48	8.76
	81.7	12.43	15.22	9.14
Refined G. N. oil	80.4	12.02	14.97	8.84
	77.4	11.78	15.23	8.66
Vanaspati, m. p., 37°C. ..	65.0	11.01	16.95	8.10
	62.4	10.67	17.10	7.85
Vanaspati, m. p., 41°C. ..	71.5	15.85	22.18	11.66
	77.2	16.89	21.89	12.42
Ghee	76.5	11.45	14.96	8.42
	84.0	11.68	13.91	8.59

Table 4: Intake, output and balance of nitrogen, phosphorus and calcium

Subject No. 1	Raw G. N. oil (Dec. 21-25, 1947)	Refined G. N. oil (Dec. 31, 1947—Jan. 4, 1948)	Vanaspati, m. p., 37°C. (Nov. 21-25, 1947)	Vanaspati, m. p., 41°C. (Dec. 11-15, 1947)	Ghee (Nov. 30 —Dec. 4, 1947)
		Nitrogen			
Food (*g.*)	28.0828	28.0828	28.0828	28.0828	28.0828
Urine (*g.*)	13.7543	16.5726	15.8330	16.7565	16.1681
Faeces (*g.*)	7.5825	9.4413	5.5078	11.1133	8.6070
Balance	6.7460	2.0689	6.7420	0.2130	3.3077

	Raw G. N. oil (Dec. 21-25, 1947)	Refined G.N. oil (Dec. 31, 1947 —Jan. 4, 1948)	Vanaspati, m.p., 37°C. (Nov. 21-25, 1947)	Vanaspati, m.p., 41°C. (Dec. 11-15, 1947)	Ghee Nov. 30— (Dec. 4, 1947)
			Phosphorus		
Food (*g.*)	2.5192	2.5192	2.5192	2.5192	2.5192
Urine (*g.*)	0.8031	0.8530	0.9812	0.9944	0.8252
Faeces (*g.*)	1.5225	1.7835	1.2812	2.2404	1.4981
Balance	0.1936	− 0.1173	0.2567	− 0.7156	0.1959
			Calcium		
Food (*g.*)	1.8108	1.8103	1.8108	1.8108	1.8108
Urine (*g.*)	0.2519	0.2115	0.3907	0.3803	0.2418
Faeces (*g.*)	1.5041	1.5375	1.8183	2.1670	0.2418
Balance	0.0548	0.0618	− 0.3982	− 0.7365	− 0.1099

Subject No. 2	Raw G.N. oil (Jan. 5-9, 1948)	Refind G. N. oil (Jan. 14-18, 1948)	Vanaspati, m.p., 37°C. (Dec. 6-10, 1947)	Vanaspati, m.p., 41°C. (Dec. 26-30, 1947)	Ghee (Dec. 16-20, 1947)
			Nitrogen		
Food (*g.*)	34.3232	34.3232	34.3232	34.3232	34.3232
Urine (*g.*)	16.0590	16.8934	15.8230	14.1581	15.9900
Faeces (*g.*)	8.1376	7.7448	12.5399	9.7506	11.0949
Balance	10.1266	9.6850	5.9603	10.4146	7.2383
			Phosphorus		
Food (*g.*)	3.0792	3.0792	3.0792	3.0792	3.0792
Urine (*g.*)	2.0764	1.9025	2.1410	2.1213	1.8593
Faeces (*g.*)	1.1893	1.1972	1.2292	1.4714	1.6069
Balance	− 0.1865	− 0.0205	− 0.2910	− 0.5135	− 0.3870
			Calcium		
Food (*g.*)	2.1232	2.2132	2.2132	2.2132	2.2132
Urine (*g.*)	0.9630	0.9086	1.0153	0.9348	0.6087
Faeces (*g.*)	1.2695	1.3350	1.9773	1.6893	1.6435
Balance	− 0.0193	− 0.0304	− 0.7794	− 1.6893	− 0.0390

Subject No. 3	Raw G. N. oil (Feb. 4-8, 1948)	Refined G.N. oil (Feb. 15-19, 1948)	Vanaspati, m.p., 37°C. (Jan. 2-6, 1948)	Vanaspati, m. p., 41°C. (Jan. 25-29, 1948)	Ghee (Jan. 14-18, 1948)
			Nitrogen		
Food (*g.*)	40.5640	40.5640	40.5640	40.5640	40.5640
Urine (*g.*)	21.5509	22.0165	20.0289	21.7774	21.2423
Faeces (*g.*)	8.7025	10.4444	10.1146	9.3891	9.1838
Balance	10.3106	8.1031	10.4205	9.3975	10.1379
			Phosphorus		
Food (*g.*)	3.6388	3.6388	3.6388	3.6388	3.6388
Urine (*g.*)	1.4435	1.4443	1.5255	1.4494	1.2379
Faeces (*g.*)	1.6736	1.8811	1.8247	1.7433	1.4714
Balance	0.5217	0.3134	0.2886	0.4461	0.9295
			Calcium		
Food (*g.*)	2.6156	2.6156	2.6156	2.6156	2.6156
Urine (*g.*)	0.7942	0.7863	0.6936	0.7858	0.5364
Faeces (*g.*)	1.5445	1.8696	1.8960	1.8245	1.8686
Balance	0.2769	0.0403	0.0260	0.0053	0.2106

Subject No. 4	Raw G.N. oil (Feb. 4-8, 1948)	Refined G.N. oil (Feb. 15-19, 1948)	Vanaspati, m.p., 37°C. (Jan. 7-11, 1948)	Vanaspati, m.p., 41°C. (Jan. 25-29, 1948)	Ghee (Jan. 15-19, 1948)
		Nitrogen			
Food (*g.*)	40.5640	40.5640	40.5640	40.5640	40.5640
Urine (*g.*)	18.8810	19.0768	18.7381	18.0039	16.6904
Faeces (*g.*)	7.6005	9.2701	7.3532	9.3044	5.9715
Balance	14.0825	12.2171	14.4727	13.2557	17.9021
		Phosphorus			
Food (*g.*)	3.6388	3.6388	3.6388	3.6388	3.6388
Urine (*g.*)	1.3258	1.3570	1.2659	1.3676	0.9897
Faeces (*g.*)	1.1711	1.4918	0.9936	1.4786	1.1011
Balance	1.1419	0.7900	1.3793	0.7926	1.5480
		Calcium			
Food (*g.*)	2.6156	2.6156	2.6156	2.6156	2.6156
Urine (*g.*)	0.5014	0.5384	0.5235	0.5203	0.3732
Faeces (*g.*)	1.1754	1.6281	1.0813	1.6299	1.2241
Balance	0.9388	0.4491	1.0108	0.4654	1.0183

Subject No. 5	Raw G.N. oil (Feb. 28—March 3, 1948)	Refined G.N. oil (Mar. 8-12, 1948)	Vanaspati, m.p., 37°C. (Jan. 27-31, 1948)	Vanaspati, m.p., 41°C. (Feb. 17-21, 1948)	Ghee (Feb. 7-11, 1948)
		Nitrogen			
Food (*g.*)	40.5640	40.5640	40.5640	40.5640	40.5640
Urine (*g.*)	19.2036	18.4077	18.8885	18.7758	18.0974
Faeces (*g.*)	9.6365	10.4444	10.1977	9.1503	7.7847
Balance	11.7239	11.7119	11.4778	12.6379	14.6819
		Phosphorus			
Food (*g.*)	3.6388	3.6388	3.6388	3.6388	3.6388
Urine (*g.*)	1.2121	1.2029	1.0772	1.3070	0.8962
Faeces (*g.*)	1.6192	1.6634	1.7196	1.6561	1.3317
Balance	0.7775	0.7725	0.8420	0.6757	1.4109
		Calcium			
Food (*g.*)	2.6156	2.6156	2.6156	2.6156	2.6156
Urine (*g.*)	0.5732	0.5308	0.5419	0.5848	0.4301
Faeces (*g.*)	1.7568	1.7994	1.8159	2.0303	1.7378
Balance	0.2856	0.2854	0.2578	0.0005	0.4377

Subject No. 6	Raw G.N. oil (Mar. 10-14, 1948)	Refined G.N. oil (Mar. 19-23, 1948)	Vanaspati, m.p., 37°C. (Feb. 6-10, 1948)	Vanaspati, m.p., 41°C. (Feb. 28—Mar 3, 1948)	Ghee (Feb. 18-22, 1948)
		Nitrogen			
Food (*g.*)	40.5640	40.5640	40.5640	40.5640	40.5640
Urine (*g.*)	18.5056	19.2940	18.1317	18.4410	18.1237
Faeces (*g.*)	9.9249	10.0671	8.1004	9.6201	9.4378
Balance	12.1335	11.2029	14.3319	12.5029	13.0025
		Phosphorus			
Food (*g.*)	3.6388	3.6388	3.6388	3.6388	3.6388
Urine (*g.*)	1.3705	1.3110	1.1857	1.2786	1.2117
Faeces (*g.*)	1.6656	1.5907	1.3836	1.7499	1.6702
Balance	0.6027	0.7371	0.0695	0.6103	0.7569
		Calcium			
Food (*g.*)	2.6156	2.6156	2.6156	2.6156	2.6156
Urine (*g.*)	0.5604	0.5604	0.6348	0.5947	0.5197
Faeces (*g.*)	1.8866	2.4246	1.5278	2.4003	2.0304
Balance	0.1686	0.3715	0.4530	0.3794	0.0655

INSTITUTION FEEDING EXPERIMENTS

SECTION I

Experiments conducted at the Aryan Orphanage, Daryagunj, Delhi.*

Feeding experiments with raw groundnut oil and vanaspati, m.p.,37 °C. were started on 15 December 1947 and continued without a break for one year. The children were medically examined every fortnight by a registered medical practitioner and the weights were taken. The experiments started with 65 boys and 52 girls and at the end of the experimental period their numbers were 43 and 25 respectively, some of the boys and girls having left the orphanage. The numbers of boys and girls under observation throughout the period were as follows:

Boys		Girls	
Vanaspati	15	Vanaspati	16
Raw groundnut oil ..	28	Raw groundnut oil	9

The general diet in the orphanage was either chappati and dal or chappati and vegetable. The children in both groups behaved similarly as regards general health.

The quantity of food taken by each child per day was approximately:

Atta or rice (oz.)	10
Dal (oz.)	$2\frac{1}{2}$
Vegetables (oz.)	6
Vanaspati or groundnut oil (oz.)	1

Groundnut oil was added while cooking either the dal or the vegetables; vanaspati was either added while cooking dal or the vegetables or was spread over the chappatis.

The weights of boys and girls of the two groups during the period of 12 months are shown in Tables 1 to 4.

Table 1: Weights of girls—raw groundnut oil group

No. of subject	Age years	Weight in lb. on: 15-12-1947	15-4-1948	13-8-1948	13-12-1948
1	8	48	51	53	57
2	18	86	95	91	82
3	14	85	95	95	104
8	11	60	65	65	68
9	9	58	60	65	67
11	14	84	89	90	95
13	15	80	85	86	..
15	11	65	74	78	83
17	9	60	61	63	65

* The work described in this section was carried out under the supervision of Drs. D.V. Karmarkar, I. S. Mathur, K. Mitra and (Miss) Radha Karnad.

Table 2: Weights of boys— raw groundnut oil group

No. of subject	Age years	Weight in lb. on: 15-12-1947	15-4-1948	13-8-1948	13-12-1948
1	11	58	58	57	64
2	10	50	51	52	53
3	11	63	64	65	65
4	12	55	60	60	61
5	8	48	50	50	52
6	11	58	60	..	60
7	12	60	61	61	64
8	10	58	59	60	64
9	9	55	54	56	58
10	10	51	51	50	52
11	12	72	75	80	83
12	14	76	74	80	..
13	16	90	95	97	..
14	12	74	78	..	..
15	12	65	68	70	..
16	13	70	77	82	87
17	12	62	66	66	70
18	12	60	64	65	70
19	16	110	120	120	118
20	13	70	..	80	..
21	15	90	102	107	..
22	13	78	85	90	94
23	13	68	72	75	74
24	13	85	95	94	95
25	19	126	130	132	133
26	16	105	110	..	107
27	14	72	78	83	87
28	14	..	105	105	..

Table 3: Weights of girls — vanaspati group

No. of subject	Age years	Weight in lb. on: 15-12-1947	15-4-1948	13-8-1948	13-12-1948
1	12	60	60	65	68
2	3	25	30	29	32
3	10	55	60	65	64
4	10	55	60	60	65
5	5	30	35	32	35
6	8	60	61	60	55
7	12	74	78	79	..
9	20	60	65	64	62
10	10	48	49	50	52
11	10	55	60	60	63
12	8	48	50	50	52
13	9	54	56	56	60
14	5	40	40	40	45
17	5	35	40	40	43
23	5	35	36	35	37
24	4	25	35	32	34

Table 4 : Weights of boys — vanaspati group

No. of subject	Age years	Weight in lb. on :			
		15-12-1947	15-4-1948	13-8-1948	13-12-1948
1	12	65	65	70	71
2	7	42	41	45	44
4	9	50	50	52	53
5	8	46	46	49	48
7	8	55	50	55	58
11	9	45	47	49	49
13	18	95	95	92	97
15	16	100	105	107	105
16	12	70	77	79	77
17	13	78	83	82	86
19	7	40	44	45	45
20	7	40	40	45	46
21	16	..	90	..	92
24	8	55	55	56	60
25	10	56	56	59	61

SECTION II

Experiments conducted at the David Sassoon Industrial School, Bombay*

Vanaspati feeding experiments on human subjects were carried out at the David Sassoon Industrial School for one year from 20 October 1947 to 20 October 1948.

There were about 400 boys in the institution but among them 190, who were going to stay for 9 months to a year, were selected. The ages of these boys ranged from 11 to 17 years.

Based on a preliminary nutrition survey, the boys were divided into two groups — normal boys and below-normal boys, and from each of these groups, two batches similar to each other as far as possible were formed by random selection. One batch from the normal group and another from the below-normal group were selected at random for vanaspati feeding and the remaining two batches were marked for raw groundnut oil feeding. The average height and weight of the four batches in the beginning are given in Table 1.

Table 1: Average heights and weights of boys at the beginning of the experiment

Group	Total No.	Av. height in.	Av. wt. lb.
A Normal boys getting oil	44	59.21	81.15
B Normal boys getting vanaspati, m.p., 37°C. ..	44	59.52	81.82
C Below-normal boys getting oil	48	58.52	76.72
D Below-normal boys getting vanaspati, m.p., 37°C. ..	49	58.58	78.61

The age distribution in the two normal batches and the two below-normal batches was practically the same (Table 2).

Table 2: Distribution according to age group

Group	Total No.	Age distribution		
		11—13	13—15	15—17
A	44	6	14	24
B	44	5	14	25
C	48	4	22	22
D	49	4	22	23

*The work described under this section was carried out by Shri P. D. Deshpande, Miss T. R. Kundaji and Miss A. M. Pavri under the supervision of Major-General S. S. Sokhey and Dr. M. V. Radhakrishna Rao. The statistical analysis of results was carried out by Smt. K. Lottikar.

Some of the boys left the school before the completion of the experiment, some absconded, two died and one dropped out. The details of the changes in the number of boys in the different groups are indicated in Table 3.

Table 3: Changes in numbers under different groups

Group	No. discharged	No. absconded	No. died	No. dropped
A	3	1	1	..
B	2	3	..	1
C	3	2	..	..
D .. .	4	2	1	..

In the beginning of the experiment, the quota of oil and vanaspati was ½ oz. per head per day. It was decided in the meeting of the Vanaspati Research Planning Committee held in Delhi on 12 December 1947 that the quota should be raised to 1 oz. per head per day. Accordingly, the boys were given 1 oz. of oil or vanaspati per head per day from 23 December 1947. Owing to the shortage of supply of oil and vanaspati, the quota was reduced to ½ oz. per head per day for the period 6 Jan. to 20 Jan. 1948. From 20 January 1948, the boys were again given their 1 oz. quota.

Oil and vanaspati were heated to a temperature of about 220° to 250°C., onion or garlic was added and the preparation was poured on cooked vegetable or dal. It was then mixed thoroughly and approximately equal amounts were distributed among boys of the same batch. The diet was served twice a day, at 12 noon and at 6.30 p.m.

The mixing of vanaspati and oil with vegetables or dal and the distribution of the food to the boys were supervised by lady inspectors at both times of feeding.

The basal diet of the experimental and the control groups was studied both in the beginning of the experiment and at the close. No great variation in the proximate composition of the diet was noticed. The two diets and their proximate composition per head per day excluding oil or vanaspati quota are given in Tables 4 and 5 respectively.

Table 4: Composition of the basal diet

	1947 oz.	1948 oz.
Cereals	12.8	13.1
Pulses	4.8	4.1
Leafy vegetables	1.5	..
Roots and tubers	7.5	3.7
Other vegetables	2.7	7.0
Milk	2.5	2.0
Sugar/jaggery	1.3	1.2
Fruits	1.6	2.0
Nuts/oil seeds	0.5	0.4

Table 5: Proximate composition of the diet

	1947	1948
Proteins (*g.*)	82.76	74.77
Fats (*g.*)	18.07	23.78
Carbohydrates (*g.*)	425.15	421.94
Calcium (*g.*)	0.58	0.56
Iron (*mg.*)	30.48	37.64
Vitamin A (*I. U.*)	2012	1249
Vitamin B_1 (*I. U.*)	605	399
Vitamin C (*mg.*)	158	153
Calories	2186	2201

The addition of 1 oz. of oil or vanaspati per head per day gave 28 g. more of fat and 252 additional calories. The diets were thus found to be low in fat, calcium and vitamin intake ; the second diet was, in addition, deficient in vitamin B_1.

The weights of the boys were taken every fortnight, and heights every month. The average gain in weight during the year and also at the end of every quarter are given in Tables 6 and 7. It can be seen from the Tables that the average heights and weights of each group had steadily increased.

Table 6: Average increase in weight (lb.)

Group	February 1948	May 1948	August 1948	October 1948
A	1.4	5.3	6.3	7.3
B	1.6	5.7	6.7	8.9
C	1.0	5.3	7.7	8.4
D	2.4	6.3	7.3	8.9

Table 7: Average increase in height (inches)

Group	February 1948	May 1948	August 1948	October 1948
A	0.34	0.74	1.19	1.61
B	0.41	0.84	1.27	1.67
C	0.29	0.78	1.41	1.77
D	0.39	0.77	1.20	1.57

All the boys gained weight during the second quarter of the year of experimentation. It may be mentioned here that there was a change of weighing machine once or twice owing to unavoidable circumstances. This may have influenced the records of weights of the boys in the second quarter and may explain the systematic gain in all the four batches during the second quarter.

Tables 8 and 9 give the average increases in weight and height during the year in the four batches.

Table 8: Average increase in weight

Group	Oil: No. observed	Oil: Av. increase in wt. lb.	Oil: Standard error	Group	Vanaspati: No. observed	Vanaspati: Av. increase in wt. lb.	Vanaspati: Standard error
A	38	7.25	±0.63	B ..	38	8.97	±0.76
C	43	8.43	±3.70	D ..	42	8.99	±0.57

Table 9: Average increase in height

Group.	Oil: No. observed	Oil: Av. increase in height in.	Oil: Standard error	Group	Vanaspati: No. observed	Vanaspati: Av. increase in height in.	Vanaspati: Standard error
A	38	1.62	±0.12	B ..	38	1.67	±0.12
C	43	1.77	±0.13	D ..	42	1.57	±0.10

Some boys suffered from malaria, influenza, etc. for short periods of 2—3 days and in some cases of even a week. The sick boys were generally given extra milk and rice after the illness and they regained the weight lost during the illness. Moreover, the losses in weight were not serious, similar losses having taken place in some boys showing no apparent illness. However, all such changes were taken into account.

A few Muslim boys — 5 in group A, 7 each in groups B and C and 8 in group D — went on Ramzan fast for about a month in July 1948. Some of them showed appreciable loss in weight. The measurements of these cases have not been omitted from consideration as there was sufficient time for them to regain the lost weight before the experiment terminated.

One boy from batch A was persistently ill during the year and was getting extra milk for the major part of the year. Another boy from batch D absconded for more than 21 months and was then brought back to the institution. These two cases have not been taken into consideration.

Nutrition surveys of boys in the different groups were carried out from time to time. Table 10 gives the percentages in the three clinical groups in each batch at the beginning and at the end of the experiment.

Table 10: Nutrition survey, percentage values

Group.		Clinical group I	Clinical group II	Clinical group III	Total
A	Oct. 30, 1947 ..	44	..	..	44
		100%	..	..	
	Oct. 16, 1948 ..	23	13	2	38
		61%	34%	5%	
B	Oct. 30, 1947 ..	44	..	..	44
		100%			
	Oct. 16, 1948 ..	23	5	9	37
		62%	14%	24%	
C	Oct. 30. 1947 ..	..	26	22	48
			54%	46%	
	Oct. 16, 1948 ..	12	20	11	43
		28%	56%	26%	
D	Oct. 30, 1947 ..	..	35	14	49
			71%	29%	
	Oct. 16, 1948 ..	27	11	4	42
		64%	26%	10%	

The experiment was terminated on 20 October 1948 and the experimental boys who were given 1 oz. of either oil or vanaspati along with their usual institutional diet during the experimental period of one year, were now given 1 oz. of groundnut oil, which was their daily intake before the experiment was started. A monthly record of height and weight measurements of these boys was maintained for six months after the experiment was over to find out the effect on growth, if any, of the change of their quota of oil and vanaspati.

A number of boys were discharged from the school during this period. Table 11 shows the number of boys in the beginning and at the end of the experiment and at the end of the six-month period after the experiment.

Table 11 : Number of boys in various groups

Group	No. of boys		
	At the beginning of the experiment *i.e.*, on 20 October 1947	At the end of the experiment, *i.e.*, on 20 October 1948	At the end of six months after the experiment, *i.e.*, on 20 April 1949
A	44	39	27
B	44	38	26
C	48	43	33
D	49	42	29

Since there was a considerable change in the number of boys in the different batches, the average height and weight of boys in the different batches on 20 October 1948 was recalculated on the basis of the height and weight of only those boys who were in the school on 20 April 1949. Table 12 gives the average height and weight on 20 October 1948 as also the increase in height and weight of these boys during the period of six months after the experiment.

Table 12 : Average weight and height of boys

Group	Av. wt. on 20 Oct. 1948 lb.	Av. increase in wt. during the period 20 Oct. 1948 to 20 April 1949* lb.	Av. height 20 Oct. 1948 in.	Av. increase in height during the period 20 Oct 1948 to 20 April 1949* in.
A	87.24	2.19 (±0.71)	60.44	0.68 (±0.08)
B	87.50	0.33 (±0.92)	59.66	0.85 (±0.09)
C	82.91	1.24 (±0.80)	60.11	0.88 (±0.09)
D	85.71	2.59 (±0.48)	59.67	0.96 (±0.10)

*Figures within brackets give the standard error.

To ascertain whether the change of dietary fat intake had affected the growth of the boys, a comparison was made of the average increase in height and weight of the boys in the same batch during the last six months of the experimental period (when they were getting 1 oz. of oil or vanaspati) with those of the six months of the post-experimental period. The results are given in Table 13.

Table 13: Effect of change of dietary fat on weight and height

Group	Av. increase in wt. during the six months before the end of the experiment* lb.	Av. increase in wt. during the six months after the end of the experiment* lb.	Av. increase in height during the six months before the end of the experiment* in.	Av. increase in height during the six months after the end of the experiment* in.
A	1.78 (±0.61)	2.19 (±0.71)	0.94 (±0.09)	0.68 (±0.08)
B	3.00 (±0.56)	0.33 (±0.92)	0.80 (±0.09)	0.85 (±0.09)
C	1.79 (±0.75)	1.24 (±0.80)	0.99 (±0.08)	0.88 (±0.09)
D	2.88 (±0.51)	2.59 (±0.48)	0.97 (±0.07)	0.96 (±0.10)

*Figures within brackets give the standard error.

It may be pointed out here that during the six-month period after the termination of the experiment, the heights and weights were taken once a month, when observations were also made regarding their general health. For the rest of the period the boys were not under direct supervision as regards their food intake or general health. Some of the boys suffered from malaria, asthma, fever, abscesses, etc. during this period. These factors may have contributed to the discrepancy in the average increase in weight of the B group.

SECTION III

Experiments conducted at the St. Philomena's Orphanage and Good Shepherd Convent, Mysore*

The children selected for the feeding trials on vanaspati and raw groundnut oil were inmates of two orphanages in Mysore. They were all below 15 years and their weights ranged from 25 to 80 lb. They were all on a poor rice diet getting less than $\frac{1}{4}$ oz. of fat per day before the commencement of the experiment. The composition of the diet given to the children is given in Table 1.

Table 1 : Composition of basal diet

Boys' Section	%	Girls' Section	%
Rice	83.0	Rice	83.5
Tur dal	2.5	Tur dal	1.8
Greengram dal	1.0	Greengram dal	0.9
Vegetables	8.0	Vegetables	7.3
Meat	0.5	Meat	0.4
Fat (raw groundnut oil) ..	1.0	Milk	0.3
Tamarind, chillies, salt, etc.	4.0	Sugar	2.5
		Fat (raw groundnut) oil ..	0.9
		Tamarind, chillies, salt, etc.	2.4

The children were divided into three groups according to their weights. Each of the weight groups was further subdivided into two groups. One group was given raw groundnut oil and the other vanaspati, m.p., 37°C. The oil or vanaspati was measured out each day for the entire group and used up as cooking medium and the cooked food was distributed, as far as possible equally, among the children so that the fat intake was maintained at the same level for all the children. Although the fat given was calculated to correspond to 5 per cent of the total solids consumed as food every day, it was found in practice that the actual fat intake ranged from 4 to 11 per cent depending on the intake of rice, which formed the bulk of the food. The average intake for the entire group may, however, be safely assumed to be about 5 per cent.

*The work described in this Section was carried out by Drs. C. R. Krishnamurthy, S. M. Bose and V. Subrahmaniyan, Indian Institute of Science, Bangalore.

It was early realised that the supplementation of the rice diet with fat had to be done by gradual steps so that the children who were receiving practically a fat-free diet could get adapted to the fat. When the fat intake was straightway raised from less than $\frac{1}{4}$ oz. per child to $\frac{4}{5}$ oz. to correspond to 5 per cent fat intake on the food taken, quite a good number of children developed diarrohea, vomitings and symptoms of nausea. The fat level had to be immediately reduced to $\frac{1}{4}$ oz. and after a few days raised to $\frac{1}{2}$ oz. Within a month by gradual increases the fat level could be raised to $\frac{4}{5}$ oz. Once the children got accustomed to the fat, they developed a taste for the food in which fat was incorporated.

The weights and heights of the children were measured every month. The children were also under constant medical observation. Individual charts for symptomatic conditions of the skin and the eyes were maintained for every child under experiment to assess vitamin deficiency, if any.

There was an unfortunate interruption of about a month in the experiments because of the unsettled political conditions in Mysore in September, 1947. Some of the children had to leave the station. As soon as they came back, they were started on $\frac{1}{2}$ oz. level and as before the level was raised to $\frac{4}{5}$ oz. in gradual doses.

In Tables 2 and 3 are given the initial and final weights of the children as also certain arbitrary markings to illustrate their clinical conditions. The marks 0, 1, 2 and 3 refer to normal, early, established and advanced cases with reference to eyes and skin. The deficiencies of the skin observed were for vitamin A (pigmentation, phrynoderma, dry and cracked face, dry and scurfy skin), riboflavin (scaly dermatitis) and niacin (thickening and desquamation of skin, bisymmetrical pigmented patches). The deficiencies of the eyes observed were for vitamin A (dry conjunctivae, xerosis and Bitot's spots) and riboflavin (corneal vascularisation). The other deficiencies observed were for riboflavin in the lips specified by angular stomatitis and dryness and cracking. The tongue was examined for glossitis, raw and red tongue and fissuring (riboflavin deficiency) and atrophic red tongue characteristic of niacin deficiency.

The deficiency scoring adopted has been verified from time to time independently by Dr. N. Purushotham, M.B.B.S. of the Ministry of Food and Agriculture ; Dr. (Miss) Pearson, Professor of Biochemistry, Women's Christian College, Madras; Dr. A. S. Ramaswamy, B. Sc., M.B.B.S., Section of Pharmacology, Indian Institute of Science ; Dr. V. R. Naidu, M.B.B.S., M.S., M.R.C.P., Professor of Pathology, University Medical College, Mysore ; and Dr. P. S. Venkatachalam, M.B.B.S., Nutrition Research Laboratories, Coonoor. Except for slight variations, all the observers were agreed about the scoring used in our experiments.

Table 2: Weight and clinical data of boys in the orphanage

Supplement given — 1 oz. raw G. N. oil or 1 oz. of vanaspati, m. p., 37°C. per head per day

Subject No.	Age years	Weight in lb.		Clinical symptoms			
		July 1947	July 1948	July 1947		July 1948	
				Eyes	Skin	Eyes	Skin
		Raw groundnut oil group					
1	12	48.00	50.00	2	1	1	1
2	9	42.00	44.50	1	1	0	0
3	11	47.25	50.25	1	2	1	2
4	9	42.50	47.50	1	1	1	2
5	12	48.00	51.25	1	1	1	1
6	11	47.25	49.50	1	1	1	1
7	9	37.25	38.50	1	1	1	1
8	7	38.50	40.75	2	1	1	0
9	6	36.25	42.12	1	1	1	1
10	5	30.50	31.25	1	1	1	1
11	10	49.50	52.75	1	0	0	0
12	12	56.00	60.75	0	0	1	3
13	12	68.00	64.00	2	1	2	2
14	12	63.00	67.00	1	1	1	0
15	11	53.50	60.00	1	0	1	0
16	13	61.75	73.00	1	0	0	0
17	9	61.25	54.25	1	1	1	0
18	13	63.50	68.50	0	0	0	0
19	12	63.50	68.00	1	0	1	0
20	12	56.50	65.50	0	1	0	0
21	13	64.00	70.50	0	0	0	1
22	13	61.50	69.75	0	0	0	0
23	11	54.50	60.50	2	0	1	0
24	11	66.50	76.00	0	1	0	1
25	12	68.50	80.50	1	0	0	1
26	13	74.50	81.25	0	1	1	0
27	15	82.75	85.50	1	0	1	1
28	14	71.75	76.50	1	1	1	2
29	15	81.50	86.00	1	0	0	2
		Vanaspati group					
1	7	48.25	53.12	1	1	0	0
2	7	35.00	37.50	1	1	1	0
3	11	47.50	50.00	2	1	0	0
4	11	49.25	57.00	0	1	1	0
5	8	48.50	51.25	2	1	2	2
6	10	46.75	53.25	0	1	1	1
7	8	31.50	40.00	1	1	1	1
8	8	39.75	42.12	1	1	1	1
9	10	42.50	48.12	0	1	0	1
10	9	45.00	49.50	0	0	0	1
11	13	65.00	72.50	1	0	1	0
12	12	58.00	62.50	1	1	3	0
13	13	62.00	70.00	0	0	0	0
14	12	56.25	60.50	2	1	3	1
15	12	62.25	68.37	1	0	0	0
16	11	58.75	65.25	1	0	0	0
17	13	62.75	68.00	0	0	0	0
18	12	64.00	74.00	1	1	1	1
19	11	54.00	58.00	2	0	1	1
20	14	72.00	83.75	0	1	1	1
21	12	71.50	81.00	1	0	1	2
22	15	73.25	84.00	1	0	0	1
23	12	57.75	69.00	2	0	1	1
24	13	68.00	74.25	0	1	0	1
25	12	68.00	76.00	1	1	0	0
26	11	67.00	75.50	2	1	2	0
27	15	80.25	94.00	1	0	0	1
28	14	74.00	84.75	0	0	0	1

Table 3: Weight and clinical data of girls in the convent

Supplement given — 1 oz. of raw G. N. oil or 1 oz. of vanaspati per head per day

Subject No.	Age years	Weight in lb.		Clinical symptoms			
		July 1947	July 1948	July 1947		July 1948	
				Eyes	Skin	Eyes	Skin
		Raw groundnut oil group					
1	9	41.00	47.00	1	0	1	0
2	10	44.75	51.00	1	0	2	0
3	11	43.25	48.50	1	1	3	1
4	10	46.00	50.25	1	1	3	1
5	9	43.75	48.25	1	1	1	1
6	12	48.00	54.00	1	1	1	0
7	8	35.25	41.50	0	1	3	1
8	9	37.00	39.25	1	0	2	0
9	8	36.50	40.50	1	1	0	1
10	8	27.00	31.25	2	0	2	1
11	4	25.75	31.00	0	1	2	1
12	11	54.75	68.00	0	0	0	0
13	12	58.50	67.25	1	1	1	0
14	12	65.00	70.25	2	0	0	0
15	13	56.50	59.75	0	0	0	0
16	12	62.50	68.25	0	0	0	1
17	14	89.25	92.00	0	0	0	1
18	15	90,00	91.25	1	0	0	0
19	15	77.25	86.00	0	1	0	0
20	13	83.25	88.75	1	0	0	0
21	12	65.25	77.25	1	1	1	1
22	14	81.25	83.50	1	0	0	0
23	12	74.75	82.25	1	0	0	0
		Vanaspati group					
1	9	41.50	50.00	1	1	0	0
2	10	46.50	53.00	0	1	0	1
3	11	46.00	47.75	1	1	1	1
4	10	35.75	39.25	3	1	2	2
5	8	37.75	41.50	1	0	1	1
6	9	35.50	38.12	1	0	2	1
7	7	36.50	34.50	2	0	1	0
8	8	32.00	36.00	2	0	2	1
9	10	26.50	32.50	3	1	3	2
10	3	21.00	26.00	0	0	1	0
11	4	24.00	27.75	1	1	0	0
12	7	27.00	29.75	1	1	0	0
13	[illegible]	29.50	33.25	1	1	1	1
14	8	43.50	50.25	1	1	1	0
15	10	37.50	47.00	1	1	1	1
16	12	62.00	67.75	0	0	0	0
17	12	53.25	59.25	1	1	1	0
18	11	62.50	72.25	1	1	0	0
19	13	61.00	70.00	1	1	1	1
20	12	63.00	66.75	0	0	0	0
21	11	58.50	71.00	0	0	0	1
22	15	81.25	87.50	0	1	0	0
23	15	70.25	78.62	1	2	0	0
24	14	90.00	92.25	1	1	0	0
25	12	68.75	79.75	1	1	0	1
26	14	84.00	98.00	1	1	0	1
27	12	67.50	81.25	2	1	0	1
28	13	81.75	90.75	1	0	0	0
29	14	74.50	88.00	1	0	0	1

METABOLISM STUDIES ON CHILDREN UNDER VANASPATI FEEDING EXPERIMENTS

As growing children are normally expected to show a better response than adults to experimental treatments affecting dietary factors, it was thought of interest to study the extent of retention and excretion of dietary fat, protein, calcium and phosphorus by children receiving a poor rice diet with vanaspati and oil supplements. These studies would also throw light on the mechanism and factors involved in the utilisation of different fats by children subsisting mainly on a poor rice diet.

With the above aim in view, six children each were selected from the oil and vanaspati groups in the girls' section. Girls were chosen mainly because they were more amenable to discipline. They were all below 11 years and their weights ranged from 45 to 60 lb. The experimental diet used was the one to which they were accustomed ever since their admission to the orphanage. The fat, however, was administered in the form of a rice preparation, locally known as Huli Anna, made by cooking rice, vegetables, salt, tamarind, chilli and other spices in oil or vanaspati as the case may be. The dietary routine of the children was as follows:

7 a.m. 1 cup (about 350 cc.) of light tea with very little milk and sugar or jaggery

9 a.m. 500—600 g. of rice and dal porridge with no fat

1 p.m. Approx. 500 g. of cooked rice preparation containing the fat

4 p.m. 1 cup of light tea

7-30 p.m. Approx. 500 g. of the same rice prepartion with fat

Before actually collecting the excretions of the children, a preliminary adaptation period of 10 days was allowed during which they were under observation. Their excretions were then collected for exactly 72 hours. Average daily intake of food was also noted. The excretions and samples of food consumed were worked up and analysed for protein, fat, calcium and phosphorus. The children were also examined for any clinical manifestations of liver enlargements. During the course of the experiment, it was realised that the daily intake of fat at $^4/_5$ oz. per day would work out to be 10—12 per cent of the dry weight of the daily intake of food. Subsequently, the metabolism studies were repeated with fat levels to correspond to 4—6 per cent. Results obtained on metabolism studies at about 10 per cent fat level are recorded in Tables 4 — 9.

Table 4: Daily intake of and daily excretions of girls below 11 years on a poor rice diet

Subject	Daily intake of dry matter	Daily excretion as faeces	Daily excretion as urine
	g.	g.	cc.
	Raw G. N. oil group		
AA	230.3	30.48	980
PL	230.3	24.20	1175
M.	230.3	23.70	1650
AP	230.3	24.70	845
PG	230.3	30.59	1290
CP	230.3	27.87	940
	Vanaspati, m.p., 37°C. group		
RR	240.8	19.7	1250
L	240.8	20.3	1730
MP	240.8	25.8	785
LRM	240.8	20.3	945
LMR	240.8	32.3	1380
M	240.8	18.8	1630

Table 5: Quantity and percentage composition of diet

	Qty consumed per day per child g.	Composition of diet %
Carbohydrate and crude fibre	184.2	79—80
Protein	23.03	10—11
Fat	24.82	10—12
Calcium	0.27	0.14
Phosphorus	2.30	1.0

Table 6: Comparative utilization of raw G.N. oil and vanaspati, m. p., 37°C.

Subject	Av. daily intake of fat g.	Av. daily excretion g.	Absorption %	Percentage of faecal fat	
				split	unsplit
		Raw G. N. oil group			
AA ..	24.82	4.35	82.5	81.4	18.6
PL ..	24.82	3.07	86.8	78.3	21.7
M. ..	24.82	2.98	88.0	79.2	20.8
AP ..	24.82	4.62	81.4	83.1	16.1
PG ..	24.82	2.83	80.9	76.5	23.5
CP ..	24.82	4.07	83.6	81.6	18.4
		Vanaspati, m.p., 37°C. group			
PR ..	25.12	4.48	82.1	78.6	21.4
L ..	25.12	5.47	78.2	73.9	26.1
MP ..	25.12	4.99	80.1	71.4	28.6
LRM ..	25.12	8.04	68.0	70.2	29.8
LMK	25.12	6.59	73.7	80.9	19.1
M ..	25.12	5.90	76.5	79.2	20.8

Table 7: Range of % absorption and % hydrolysis of raw G.N. oil and vanaspati

Fat	Percentage absorption			No. of observations
	lowest	highest	average	
Raw G.N. oil ..	81.4	88.6	85.0	6
Vanaspati, m. p., 37°C. ..	73.7	82.1	77.9	6
	Percentage hydrolysis of faecal fat			
	lowest	highest	average	
Raw G. N. oil ..	76.5	83.1	79.8	6
Vanaspati, m.p., 37°C...	70.2	80.9	75.5	6

Table 8: Comparative utilization of nitrogen

Subject	Av. daily intake of N g.	Av. daily output of N: urine g.	faeces g.	total g.	Av. retention g.
		Raw G. N. oil group			
AA ..	3.487	1.547	1.725	3.272	+0.215
PL ..	3.487	1.376	1.147	2.523	+0.964
M ..	3.487	1.320	1.271	2.591	+0.896
AP ..	3.487	1.405	1.181	2.586	+0.901
PG ..	3.487	1.901	1.418	3.319	+0.168
CP ..	3.487	1.812	0.841	2.653	+0.834
		Vanaspati, m.p., 37°C. group			
PR ..	3.512	1.497	1.070	2.567	+ 0.945
L ..	3.512	1.958	1.412	3.370	+ 0.142
MP ..	3.512	1.320	1.862	2.182	+ 0.330
LRM ..	3.512	1.646	1.517	3.163	+ 0.349
LMK	3.512	0.851	2.314	3.165	+ 0.351
M ..	3.512	1.814	1.327	3.141	+ 0.371

Table 9: Effect of raw G.N. oil and vanaspati on Ca & P metabolism

Subject	Calcium: Av. daily intake mg.	Av. daily output urine mg.	faeces mg.	total mg.	Balance mg.	Phosphorus: Av. daily intake mg.	Av. daily output urine mg.	faeces mg.	total mg.	Balance mg.
			Raw G. N. oil group							
AA ..	364	94.5	182.7	277.2	+86.8	1405	698	194	892	+513
PL ..	364	29.3	101.4	130.7	+233.6	1405	665	148	830	+592
M ..	364	139.5	192.9	332.4	+31.8	1405	665	126	791	+614
AP ..	364	54.0	208.4	262.4	+101.8	1405	554	132	686	+719
PG ..	364	72.0	175.9	247.0	+116.3	1405	574	332	906	+499
CP ..	364	94.5	156.9	251.4	+112.8	1405	577	297	864	+541
			Vanaspati, m.p., 37°C. group							
PR ..	328.6	22.5	170.5	193.0	+135.6	1797	722	341	1063	+734
L ..	328.6	76.5	178.5	255.0	+73.6	1797	815	341	1156	+641
MP ..	328.6	108.0	193.8	301.8	+26.8	1797	672	374	1046	+751
LRM	328.6	85.5	154.0	240.4	+88.2	1797	651	309	960	+837
LMK	328.6	49.5	296.2	345.7	+17.1	1797	524	468	992	+805
M ..	328.6	58.5	182.5	241.0	+87.6	1797	771	141	912	+885

The metabolism studies were repeated twice on the same experimental subjects at 5% fat level, once in April 1948 and later in August 1948. The results obtained are embodied in Tables 10 — 13.

Table 10: Quantity and percentage composition of diet

	April 1948		August 1948	
	Qty consumed by each girl per day *g.*	Composition °/₀	Qty consumed by each girl per day *g.*	composition °/₀
Carbohydrate and crude fibre	198.60	84.80	201.32	84.43
Protein	22.02	9.39	23.11	9.68
Fat	12.41	5.29	12.41	5.21
Calcium ..	0.29	0.124	0.31	0.13
Phosphorus ..	1.18	0.503	1.27	0.54

Table 11: Comparative utilisation of raw G. N. oil and vanaspati, m.p., 37°C.

Subject	Daily intake of fat *g.*	Daily excretion *g.*	Absorption °/₀	Percentage of faecal fat split	unsplit
		Raw G. N. oil group			
April 1948					
PL ..	12.41	1.31	89.5	84.6	15.4
M ..	12.41	1.09	91.2	89.2	10.8
AP ..	12.41	1.08	91.3	88.4	11.6
PG ..	12.41	1.29	90.4	88.1	11.9
CP ..	12.41	0.96	92.3	89.2	10.8
August 1948					
PL ..	12.41	1.40	88.7	83.7	16.3
M ..	12.41	1.32	89.4	86.2	13.8
AP ..	12.41	1.19	90.4	89.4	10.6
PG ..	12.41	1.08	91.3	89.8	10.2
CP ..	12.41	0.91	92.7	89.8	10.2
		Vanaspati, m.p., 37°C. group			
April 1948					
PR ..	12.41	1.65	86.7	82.1	17.9
MR ..	12.41	1.47	88.2	81.7	18.3
LRM ..	12.41	1.32	89.4	83.8	16.2
LMK ..	12.41	1.38	88.9	84.6	15.4
M ..	12.41	1.22	90.2	87.2	12.8
August 1948					
PR ..	12.41	1.56	87.4	83.4	16.6
MR ..	12.41	1.44	88.4	83.8	16.2
LRM ..	12.41	1.40	88.7	82.7	17.3
LMK ..	12.41	1.69	86.4	86.4	13.6
M ..	12.41	1.33	89.3	85.4	14.6

Table 12: Comparative utilisation of dietary nitrogen

Subject	Av. daily intake of N *g.*	Av. daily output of N			Av. retention *g.*
		urine *g.*	faeces *g.*	total *g.*	
		Raw G. N. oil group			
April 1948					
PL ..	3.334	1.294	1.562	2.856	+0.478
M ..	3.334	1.212	1.197	2.409	+0.925
AP ..	3.334	1.347	1.186	2.533	+0.801
PG ..	3.334	1.268	1.212	2.480	+0.854
CP ..	3.334	1.645	1.372	3.017	+0.317
August 1948					
PL ..	3.491	1.478	1.729	3.207	+0.284
M ..	3.491	1.624	1.398	3.022	+0.469
AP ..	3.491	1.494	1.376	2.870	+0.621
PG ..	3.491	1.673	1.293	2.966	0.525
CP ..	3.491	1.423	1.224	2.647	+0.844
		Vanaspati, m.p., 37°C. group			
April 1948					
PR ..	3.334	1.497	1.379	2.876	+0.458
MR ..	3.334	1.486	1.043	2.529	+0.805
LRM ..	3.334	1.432	1.089	2.511	+0.823
LMK ..	3.334	1.643	0.832	2.475	+0.859
M ..	3.334	1.578	0.699	2.677	+0.657
August 1948					
PR ..	3.491	1.583	1.427	2.810	+0.681
MR ..	3.491	1.479	1.371	2.850	+0.641
LRM ..	3.491	1.638	1.363	2.801	+0.690
LMK ..	3.491	1.642	1.398	2.840	+0.651
M	3.491	1.449	1.284	2.733	+0.758

Table 13: Effect of raw G. N, oil and vanaspati on Ca and P metabolism

Subject	Calcium Daily intake mg.	Calcium Daily output urine mg.	Calcium Daily output faeces mg.	Calcium Daily output total mg.	Calcium Retention mg.	Phosphorus Daily intke mg.	Phosphorus Daily output urine mg.	Phosphorus Daily output faeces mg.	Phosphorus Daily output total mg.	Phosphorus Retention mg.
Raw G. N. oil group										
April 1948										
PL ..	290	82	176	258	+32	1180	698	278	976	+204
M ..	290	87	170	257	+33	1180	746	283	1029	+151
AP ..	290	73	181	254	+34	1180	739	297	1036	+144
PG ..	290	92	179	271	+19	1180	1028	186	1214	−34
CP ..	290	109	169	278	+12	1180	785	184	969	+211
August 1948										
PL ..	310	87	178	265	+45	1270	782	347	1129	+141
M ..	310	89	181	270	+40	1270	797	427	1224	+46
AP ..	310	94	203	297	+13	1270	814	337	1251	+19
PG ..	310	79	180	259	+51	1270	827	391	1218	+52
CP ..	310	78	176	254	+56	1270	784	420	1204	+66
Vanaspati, m.p., 37°C. group										
April 1948										
PR ..	290	87	183	270	+20	1180	642	369	1011	+169
MR ..	290	89	191	280	+10	1180	657	392	1049	+131
LRM	290	74	184	258	+32	1180	651	401	1052	+182
LMK	290	91	182	273	+17	1180	673	381	1054	+126
M ..	290	90	189	279	+11	1180	810	209	1019	+61
August 1948										
PR ..	310	89	174	263	+47	1270	784	384	1168	+102
MR ..	310	89	179	268	+42	1270	792	378	1170	+100
LRM ..	310	92	181	273	+37	1270	797	364	1161	+109
LMK	310	98	183	281	+29	1270	805	372	1177	+93
M ..	310	93	192	285	+25	1270	810	373	1183	+87

Table 14: Percentage retention of nitrogen, calcium and phosphorus and digestibility of fat at two different levels of fat ingestion

Details	Raw G.N. oil		Vanaspati, m.p., 37°C.	
	10—12%	5—6%	10—12%	5—6%
Digestibility of fat	85.0	90.9	81.0	88.4
% retention of N	19.0	20.2	18.2	18.1
% retention of Ca	31.3	12.1	30.2	11.6
% retention of P	32.3	14.4	32.8	10.3

By July 1948, the feeding experiments on orphanage children in Mysore completed one year. Since some more children had been brought under experiment a few months after its commencement in July 1947, it was thought desirable to continue the feeding of oil and vanaspati till September 1948. On October 1, 1948 the children were put back on their original fat-deficient diet. The clinical observations and height and weight measurements were, however, continued up to the end of March 1949. The results of weight measurements for this post-feeding period of 6 months are given in Table 15 as the computed averages.

Table 15: Weight increments of orphanage children under 15 years on rice diet + 5 - 10 % fat

Group	Raw G.N. oil			Vanaspati, m.p., 37°C.		
	Initial weight lb.	Increment in 12 months experimental period	Increment in 6 months post-experimental period	Initial weight lb.	Increment in 12 months experimental period	Increment in 6 months post-experimental period
Boys						
I ..	42.25	2.81±0.23	1.2±0.12	43.40	4.35±0.62	2.90±0.32
II ..	62.57	5.45±0.39	2.2±0.31	61.43	6.93±0.32	4.40±0.61
III ..	77.63	4.69±0.45	4.0±0.10	74.20	11.10±0.32	5.0±0.46
Girls						
I ..	38.93	4.93±0.34	1.4±0.1	34.70	4.39±0.38	1.5±0.12
II ..	59.45	7.25±0.92	2.5±0.3	60.04	7.63±0.67	2.7±0.61
III ..	80.14	5.71±0.61	3.5±0.6	77.25	8.52±0.40	3.8±0.27

Group I, under 50 lb.; group II, 50-70 lb.; group III, above 70 lb.

STATISTICAL ANALYSIS*

Section I —Animal Experiments

A—GAIN IN LIVE WEIGHT

(*i*) INDIAN DAIRY RESEARCH INSTITUTE, BANGALORE

Average weekly gains in live weight of each group of 6 rats are shown in Table 1.

Table 1: Average weekly gain (in g.) in live weight

	Synthetic diet	Poor rice diet	Poor rice diet supplemented with vitamins	Poor rice diet supplemented with 0.3 % $CaCO_3$	Poor rice diet supplemented with 7 % casein	Poor Bengali diet (A)	All diets
				Males			
Ghee	11.6	4.7	.. 7.2	5.8	8.8	7.6	7.6
Raw groundnut oil	10.9	4.2	6.5	5.0	7.8	6.3	6.8
Refined groundnut oil ..	11.6	4.1	6.9	5.1	7.9	6.2	7.0
Vanaspati, m.p., 37°C... ..	10.6	4.0	7.1	4.5	7.0	5.2	6.4
Vanaspati, m.p., 41°C.	11.2	4.2	7.6	4.5	7.6	7.1	7.0
All fats ..	11.2	4.2	7.0	5.0	7.8	6.5	7.0
				Females			
Ghee	7.0	3.2	5.7	5.2	6.6	6.5	5.7
Raw groundnut oil	7.2	3.3	4.9	5.3	6.1	6.1	5.5
Refined groundnut oil ..	7.1	3.4	5.6	4.0	6.0	6.0	5.3
Vanaspati, m.p., 37°C.	6.7	3.2	6.0	4.5	5.9	5.9	5.4
Vanaspati, m.p., 41°C.	7.0	3.5	6.0	4.8	5.7	6.7	5.6
All fats ..	7.0	3.3	5.6	4.8	6.1	6.3	5.5

The initial weights of rats in this experiment showed considerable variation. The correlation between initial weight of a rat and its average gain in live weight was, however, not found to be statistically of any significance. The within group coefficient of correlation between the two factor was only 0.057.

*The statistical analysis of the data collected by the Vanaspati Research Planning Committee was carried out by Prof. S. Swaroop, All-India Institute of Hygiene and Public Health, Calcutta.

Table 2 summarises the results of the analysis of variance on figures of gains in live weight.

Table 2: Analysis of variance of average weekly gain in live weight

Source of variation	Degree of freedom	Sum of squares	Mean squares	F	Expected values of F at the probability level of: 5 %	1 %
Fats	4	23.65	5.192	6.24	2.37	3.32
Sexes	1	189.95	189.950	200.37	3.84	6.64
Diets	5	992.53	198.506	209.39	2.21	3.02
Fat × sex	4	8.50	2.125	2.24	2.37	3.32
Fat × diet	20	27.98	1.399	1.48	1.60	1.98
Sex × diet	5	166.66	33.332	35.16	2.21	3.02
Fat × sex × diet ..	20	7.38	0.369	<1	1.60	1.98
Error	300	284.29	0.948			
Total ..	359	1700.94				

In this experiment also fats are found to be associated with significantly different rates of growth, the highest figure being for ghee. Only in the case of male rats, vanaspati, m.p., 37°C. has the lowest growth rate. Among female rats the growth does not appear to vary for different fats.

(*ii*) Indian Institute of Science, Bangalore

Table 3 summarises the average weekly gains in live weight per rat separately for sex, diet and fat groups over a period of 12 weeks.

Table 3: Average weekly (in g.) gain in live weight

	Synthetic diet	Poor rice diet	Poor riee diet supplemented with vitamins	Poor rice diet supplemented with 0.3% $CaCO_3$	Poor rice diet supplemented with 7% casein	Poor Bengali diet (A)	All diets
				Males			
Ghee	11.1	5.0	6.6	4.8	6.6	11.6	7.6
Raw groundnut oil	11.8	5.0	5.6	4.6	5.7	11.1	7.3
Refined groundnut oil ..	10.4	5.1	6.2	4.6	6.3	10.7	7.2
Vanaspati, m.p., 37°C. ..	9.4	5.2	6.0	5.2	5.9	11.4	7.2
Vanaspati, m.p., 41°C... ..	11.7	4.3	6.0	4.6	5.3	11.5	7.3
All fats ..	10.9	4.9	6.1	4.8	6.0	11.3	7.3
				Females			
Ghee	8.7	3.9	5.5	4.6	5.2	9.2	6.2
Raw groundnut oil	8.7	4.8	5.2	4.0	5.6	8.5	6.1
Refined groundnut oil ..	8.5	4.0	5.1	4.0	5.9	9.1	6.1
Vanaspati, m.p., 37°C. ..	8.1	4.6	5.4	4.5	4.7	9.2	6.1
Vanaspati, m.p.. 41°C. ..	7.7	4.1	4.8	4.1	4.6	9.1	5.7
All fats ..	8.3	4.3	5.2	4.2	5.2	9.0	6.0

The initial weights of rats were practically the same in this experiment.

All the same, an examination was made as to whether the initial weight was correlated with subsequent gain in live weight. The within regression coefficient of gain in weight on initial weight was found to be 1.403, a significant figure compared with its standard error of 0.25. The analysis of data relating to gain in live weight was, therefore, carried out with due allowance for initial weight.

It would appear that both among male and female rats the highest increase in weight was observed in the case of ghee. The statistical significance of the effect of different factors, viz. the fat administered, sex, or the type of diet, on the increase in weight has been analysed statistically by the analysis of variance. The results are given in Table 4. By this analysis it is also possible to examine whether different fats reacted in a similar manner or differently in the presence or absence of various diets.

Table 4: Analysis of variance of average weekly gain in live weight

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F. observed	Expected value of F at probability level of: 5%	1%
Fats	4	5.414	1.354	8.31	2.37	3.32
Sexes	1	128.735	128.735	789.79	3.84	6.64
Diets	5	1964.056	392.811	2409.88	2.21	3.02
Fat × sex	4	3.166	0.792	4.86	2.37	3.32
Fat × diet	20	33.437	1.672	10.26	1.60	1.98
Sex × diet	5	55.032	11.006	67.52	2.21	3.02
Fat × sex × diet ..	20	20.516	1.026	6.29	1.60	1.98
Error	300	48.711	0.163			
Total ..	358	2259.067				

The following conclusions are drawn in regard to the relative values of different fats:

(*i*) Different fats show statistically significant differences in regard to growth rates in rats. The highest growth has been recorded in the case of ghee for both males and females. In the case of female rats the lowest value is observed for vanaspati, m.p., 41°C.

(*ii*) Different fats react in a statistically significantly different manner in conjunction with the several series of diets.

(*iii*) Different fats appear to show a tendency to vary in their effect in respect of the two sexes.

From the combined result of all the diets it is seen that the average growth rate is highest for ghee and lowest for vanaspati, m. p., 41°C., the difference being statistically significant. But as the interaction between diet and fats is also significant, a further conclusion is that the poor result with vanaspati, m. p., 41°C. is more marked in conjunction with poor rice diet (with or without supplements) than with synthetic diet or modified poor Bengali diet.

iii. Indian Veterinary Research Institute, Izatnagar

Table 5 summarises the average gains in live weight of each group of six rats.

Table 5: Average weekly gain (in g.) in live weight

	Synthetic diet	Poor rice diet	Poor rice diet supplemented with vitamins	Poor rice diet supplemented with 0.3% $CaCO_3$	Poor rice diet supplemented with 7% casein	Poor rice diet supplemented with vitamins, $CaCO_3$, and casein	All diets
				Males			
Ghee	14.7	4.5	13.1	6.7	3.9	13.6	9.4
Raw groundnut oil	13.6	3.1	12.2	4.0	3.2	14.8	8.5
Refined groundnut oil	13.4	3.1	10.9	4.1	3.1	15.0	8.3
Vanaspati, m. p., 37°C.	13.1	3.0	12.4	4.0	3.7	15.7	8.7
Vanaspati, m. p., 41°C.	13.9	3.1	12.5	3.4	4.0	16.1	8.8
All fats	13.7	3.4	12.2	4.4	3.6	15.1	8.7
				Females			
Ghee	7.9	4.3	9.6	5.4	5.0	8.2	6.7
Raw groundnut oil	8.0	3.0	8.3	4.7	4.6	8.2	6.1
Refined groundnut oil	8.5	2.7	8.1	4.6	4.5	9.3	6.3
Vanaspati, m. p., 37°C.	8.1	3.6	9.3	4.0	3.7	8.3	6.1
Vanaspati, m. p., 41°C.	8.2	3.7	8.0	4.8	4.7	9.1	6.4
All fats	8.1	3.4	8.6	4.7	4.5	8.6	6.3

As the initial weights of rats differed from group to group, an analysis of co-variance was carried out but the within co-variance is found to be statistically insignificant.

A straightforward analysis of variance was, therefore, carried out and the results are given in Table 6,

Table 6: Analysis of variance of average weekly gain in live weight

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F observed	Value of F at probability level of: 5 %	1 %
Fats	4	31.20	7.800	6.76	2.37	3.32
Sexes	1	514.51	514.510	446.24	3.84	6.64
Diets	5	4589.97	917.994	796.18	2.21	3.02
Fat × sex	4	4.59	1.148	<1	2.37	3.32
Fat × diet	20	72.85	3.642	3.16	1.60	1.98
Sex × diet	5	783.86	156.772	135.97	2.21	3.02
Fat × sex × diet ..	20	33.91	1.696	1.47	1.60	1.98
Error	300	345.89	1.153			
Total ..	359	6376.78				

The conclusions to be drawn from this analysis are:

(1) Fats are significantly different from each other as judged by average gain in live weight. Average gain in weight is highest for ghee and lowest for refined groundnut oil in the case of males.

(2) The fats react differently in different diets.

(iv) University College of Science and Technology, Calcutta.

The average gain in live weight of each group of 6 rats is shown in Table 7.

Table 7: Average weekly gain (in g.) in live weight

	Synthetic diet	Poor rice diet	Poor rice diet supplemented with vitamins	Poor rice diet supplemented with 0.3% $CoCO_3$	Poor rice diet supplemented with 7% casein	Poor Bengali diet (A)	All diets
				Males			
Ghee	8.0	3.6	10.0	7.9	7.3	6.6	7.2
Raw groundnut oil ..	7.0	3.3	5.5	6.1	5.7	6.4	5.7
Refined groundnut oil ..	6.7	3.1	7.9	7.3	5.2	6.6	6.1
Vanaspati, m.p., 37 C.	7.3	4.0	8.8	6.2	6.3	5.8	6.4
Vanaspati, m. p., 41°C.	8.4	4.2	7.5	6.2	6.7	6.8	6.6
All fats ..	7.5	3.6	7.9	6.8	6.2	6.4	6.4
				Females			
Ghee	8.0	3.7	7.0	6.9	7.3	5.1	6.3
Raw groundnut oil ..	7.6	2.9	5.3	5.0	5.0	5.5	5.3
Refined groundnut oil..	6.9	2.7	7.5	5.9	5.9	5.2	5.7
Vanaspati, m. p., 37°C.	6.8	4.3	7.2	5.3	6.5	5.8	6.0
Vanaspati, m. p. ,41 C.	7.8	3.7	7.1	6.1	6.9	5.6	6.2
All fats ..	7.4	3.5	6.8	5.9	6.3	5.4	5.9

For this centre also the initial weight of rats was not found to be correlated with the gain in live weight.

Table 8 summarises the results.

Table 8: Analysis of variance of average weekly gain in live weight

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F	Expected value of F at the probability level of: 5 %	1 %
Fats	4	73.30	18.325	20.45	2.37	3.32
Sexes	1	25.11	25.110	28.02	3.84	6.62
Diets	5	605.67	121.134	135.19	2.21	3.04
Fat × sex	4	3.37	0.842	<1	2.37	3.32
Fat × diet	20	84.93	4.426	4.74	1.60	1.98
Sex × diet	5	20.78	4.156	4.64	2.21	3.02
Fat × sex × diet ..	20	26.55	1.328	1.48	1.60	1.98
Error	300	268.68	0.896			
Total ..	359	1108.39				

Once again ghee has the highest growth rate. The lowest growth rate was obtained in the case of raw groundnut oil. A significant interaction between fats and diets indicates that fats react in different manners in the presence of various diets.

(v) Analysis of the combined data of the four centres

In the foregoing analysis the experimental data have been studied separately for each centre. By the technique of analysis of variance it is possible to examine the inter-centre differences and at the same time arrive at conclusions based on the combined figures of all the centres. We can allow for variation attributable separately to differences in centres, sexes, diets and fats. At the same time, the variation arising through the interaction of any 2 or 3 or 4 of these factors is separately estimated. Although in all the 4 centres, 6 or more different diets were experimented upon only 5 diets were common to all the centres. The diets were :

1. Synthetic diet
2. Poor rice diet
3. Poor rice diet supplemented with vitamins
4. Poor rice diet supplemented with calcium carbonate
5. Poor rice diet supplemented with casein

Table 9 summarises the significance of data relating to the 4 centres, 5 diets, 5 fats and 2 sexes.

Table 9: Analysis of variance of combined data on gain in live weight

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F	Expected value of F at probability level of: 5 %	1 %
Fats (F)	4	97.792	24.448	29.92	2.38	3.34
Sexes (S)	1	431.750	431.750	528.46	3.85	6.66
Diets (D)	4	4345.136	108.6284	132.96	2.38	3.34
Centres (C)	3	76.855	25.618	31.36	2.61	3.80
Interaction						
Fat × sex	4	10.142	2.536	3.10	2.38	3.34
Fat × diet	16	59.160	3.694	4.52	1.65	2.01
Fat × centre	12	66.167	5.514	6.75	1.76	2.20
Sex × diet	4	362.254	90.564	110.85	2.38	3.34
Sex × centre	3	74.458	24.819	30.38	2.61	3.80
Diet × centre ..	12	1560.346	130.029	159.15	1.76	2.20
Fat × sex × diet ..	16	16.974	1.061	1.30	1.65	2.01
Fat × sex × centre	12	7.024	0.585	<1	1.76	2.20
Fat × diet × centre	48	85.390	1.779	2.18	1.36	1.54
Sex × diet × centre	12	326.813	27.234	33.33	1.76	2.20
F × C × S × D ..	48	53.723	1.119	1.37	1.36	1.54
Error	1000	817.378	0.817			
Total ..	1199	8391.308				

The following main conclusions are to be drawn from the tabulated results :

(*i*) It is statistically established that the fats are not alike in producing increase in live weight.

(*ii*) There is appreciable difference in the relative effect of fats in relation to diet, sex or centre.

This analysis of variance also shows a high degree of significance of differences between diets and sexes.

If the average gain in live weight is studied for each fat, it is seen that the highest gain in live weight is recorded in the case of ghee.

	Av. gain in live weight g.
1. Ghee.	6.82
2. Raw groundnut oil	6.02
3. Refined groundnut oil	6.09
4. Vanaspati, m. p., 37°C.	6.15
5. Vanaspati m. p., 41°C.	6.26

With the exception of ghee the other 4 fats show a relatively smaller degree of variation. It is of interest to see whether the remaining 4 fats show any statisically significant differences among themselves in respect

of growth rates. A four variate analysis of variance was tried on the data after excluding ghee for all the 4 centres. The results are given in Table 10.

Table 10: Analysis of variance of average gain in live weight for all fats excluding ghee

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F	Expected value of F at probability level of: 5 %	1 %
Fats (F)	3	7.351	2.450	2.97	2.61	3.80
Sexes (S)	1	295.880	295.880	359.08	3.85	6.66
Diets (D)	4	3500.970	875.243	1062.19	2.38	3.34
Centres (C)	3	40.807	13.602	16.51	2.61	3.80
Interaction						
Fat × sex	3	0.568	0.189	<1.00	2.61	3.80
Fat × diet	12	46.128	3.844	4.67	1.76	2.20
Fat × centre ..	9	45.212	5.024	6.10	1.89	2.43
Sex × diet	4	286.999	71.750	87.08	2.38	3.34
Sex × centre.. ..	3	56.268	18.756	22.76	2.61	3.80
Diet × centre ..	12	1227.102	102.259	124.10	1.76	2.20
Fat × sex × diet ..	12	16.151	1.346	1.63	1.76	2.20
Fat × sex × centre	9	5.251	0.583	<1.00	1.89	2.43
Fat × diet × centre	36	64.961	1.804	2.19	1.44	1.66
Sex × diet × centre	12	252.388	21.032	25.52	1.76	2.20
F × S × D × C ..	36	33.796	0.939	1.14	1.44	2.43
Error	800	659.254	0.824			
Total ..	959	6539.086				

The differences between fats are now significant only at the 5 per cent probability level of significance and not at 1 per cent. Table 11 shows separately for each centre the average weekly gain in live weight for individual fats.

Table 11: Average weekly gain in live weight at different centres

	Indian Dairy Research Institute	Indian Institute of Science	Indian Veterinary Research Institute	University College of Science
Ghee	6.58	6.20	7.51	6.98
Raw groundnut oil ..	6.13	6.10	6.47	5.37
Refined groundnut oil ..	6.16	6.00	6.30	5.91
Vanaspati, m.p., 37° C. ..	5.95	5.90	6.48	6.26
Vanaspati, m.p., 41° C. ..	6.20	5.72	6.63	6.48

If the figures for ghee are excluded from the discussion, vanaspati, m.p., 41°C. shows the lowest rate of increase in the case of Indian Institute of Science and the same fat shows the highest rate of growth for the remaining three centres.

B—GAIN IN LIVE WEIGHT OF RATS OF SECOND AND THIRD GENERATIONS

Experiments on the growth of rats in the second and third generations put on the experimental fats were carried out in all the four centres. But as adequate numbers of experimental rats for the second and third generations were not uniformly available and as quite a number of them died

before the completion of the experimental period, the data available from two of the centres, viz. the Indian Dairy Research Institute, Bangalore, and the College of Science, Calcutta, are far from complete. The data from the remaining two centres have been analysed.

Table 12 gives the average weekly gain in live weight in the two centres in the second and third generations.

Table 12 : Average weekly gain (in g.) in live weight

Diets	Ghee		Raw G.N. oil		Refined G.N. oil		Vanaspati, m. p., 37 C.		Vanaspati, m. p., 41 C.		All fats.	
	M*	F*	M	F	M	F	M	F	M	F	M	F
Second generation												
Indian Institute of Science, Bangalore												
Sr. I.	12.0	8.2	11.4	6.6	10.2	6.6	11.4	8.3	10.5	7.5	11.1	7.4
Sr. IV(a)	9.5	6.7	9.5	6.7	8.3	6.4	7.6	6.8	7.9	6.9	8.6	6.7
All diets	10.8	7.5	10.4	6.6	9.3	6.5	9.5	7.5	9.2	7.2	9.8	7.0
Indian Veterinary Research Institute, Izatnagar												
Sr. II	5.2	4.3	4.1	4.2	3.8	4.4	4.0	4.7	3.4	3.2	4.1	4.1
Sr. III	10.2	7.8	9.3	7.0	8.8	6.9	9.2	7.3	8.4	6.9	9.2	7.2
Sr. IV	5.2	5.2	5.2	5.6	4.5	5.5	4.0	4.6	3.5	5.5	4.5	5.3
Sr. V	5.8	5.7	6.1	6.0	6.2	5.8	5.9	5.4	4.5	5.7	5.7	5.7
Sr. V(a)	13.3	8.8	13.5	9.5	12.7	9.3	12.7	9.6	13.7	8.9	13.2	9.2
All diets	7.9	6.4	7.6	6.5	7.2	6.4	7.2	6.3	6.7	6.0	7.3	6.3
Third generation												
Indian Institute of Science, Bangalore.												
Sr. I.	11.7	9.9	12.1	9.3	12.7	9.2	12.2	9.5	12.0	9.8	12.2	9.6
Sr. IV(a)	8.7	7.0	8.5	6.6	8.5	6.3	9.3	6.5	8.6	5.9	8.7	6.4
All diets	10.2	8.5	10.3	7.9	10.6	7.8	10.8	8.0	10.3	7.9	10.4	8.0
Indian Veterinary Research Institute, Izatnagar												
Sr. II ..	4.4	4.4	3.7	3.9	3.2	2.9	3.4	3.9	3.8	4.3	3.7	3.9
Sr. III	12.2	9.2	11.7	8.0	11.5	7.0	12.9	9.2	11.3	7.9	11.9	8.2
Sr. IV	6.3	6.0	4.6	5.3	5.5	5.3	4.9	5.8	4.9	5.4	5.2	5.5
Sr. V ..	6.6	5.9	5.7	5.0	6.1	5.0	6.4	5.5	6.8	5.9	6.3	5.5
Sr. V(a)	13.7	9.7	14.3	9.8	12.9	9.0	13.8	10.0	12.7	9.1	13.5	9.5
All diets	8.7	7.0	8.0	6.4	7.8	5.8	8.3	6.9	7.9	6.5	8.1	6.5

*M—males; F—females

The initial weights of the rats in the second and third generations varied considerably from rat to rat and also from one group of rats to another. The within group regression of the growth on the initial weight was, therefore, worked out and whenever this relationship emerged into statistical significance, due allowance in the growth was made for differences in initial weight.

As the diets varied in number from one centre to another, separate analyses are presented for each centre.

Table 13: Analysis of variance of weekly gain in weight of rats of second generation at the Indian Veterinary Research Institute, Izatnagar

Source of variation	Including ghee				Excluding ghee			
	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Degrees of freedom	Sum of squares	Mean squares	F (observed)
Fats	4	22.42	5.61	6.10*	3	14.68	4.893	5.88*
Sexes	1	76.44	76.44	83.09*	1	45.92	45.929	55.19*
Diets	4	1993.23	498.31	541.64*	4	1636.57	409.143	491.76*
Fat × sex	4	7.69	1.92	2.09	3	2.24	0.746	1
Fat × diet	16	23.07	1.44	1.57	12	13.87	1.156	1.39
Sex × diet	4	227.03	56.76	61.70*	4	186.46	46.615	56.03*
F × S × D	16	16.62	1.04	1.13	12	15.23	1.269	1.53
Error	250	229.95	0.92		200	166.32	0.832	
Total	299	2569.45			239	2081.29		

* Significant at 1% level.

Within regression of increase in weight on initial weight (—0.199) is not of statistical significance.

Table 14: Analysis of variance of weekly gain in live weight of rats of the third generation at the Indian Veterinary Research Institute, Izatnagar

Source of variation	Including ghee				Excluding ghee			
	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Degrees of freedom	Sum of squares	Mean squares	F (observed)
Fats	4	36.04	9.01	9.87*	3	15.96	5.32	7.65*
Sexes	1	193.56	193.56	212.00*	1	154.12	154.12	221.76*
Diets	4	2584.82	646.20	707.78*	4	2104.85	526.21	757.14*
Fat × sex	4	3.99	1.00	1.10	3	3.99	1.00	1.44
Fat × diet	16	37.23	2.33	2.55*	12	34.21	2.85	4.10*
Sex × diet	4	260.87	65.22	71.43*	4	225.55	56.39	81.14*
F × S × D	16	6.46	0.40	<1	12	3.26	0.27	<1
Error	250	228.13	0.91		200	138.91	0.69	
Total	299	3351.10			239	2680.85		

*Significant at 1% level

Within regression of increase in weight on initial weight (—0.024) is not of statistical significance.

Table 15: Analysis of varianance of weekly gain in live weight of rats of second generation at the Indian Institute of Science, Bangalore

Source of variation	Including ghee				Excluding ghee			
	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Degrees of freedom	Sum of squares	Mean squares	F (observed)
Fats (F)	4	16.06	4.015	24.04*	3	13.65	4.55	27.4*
Sexes (S)	1	179.28	179.280	1073.53*	1	127.15	127.15	766.0*
Diets (D)	1	33.26	33.260	199.16*	1	32.66	32.66	197.0*
Fat × sex	4	20.74	5.185	31.05*	3	17.62	5.87	35.4*
Fat × diet	4	7.69	1.922	11.51*	3	6.25	2.68	12.5*
Sex × diet	1	14.07	14.070	84.25*	1	8.76	8.76	52.8*
F × S × D	4	1.40	0.350	2.10	3	5.07	1.69	10.2*
Error	89	14.83	0.167		72	11.96	0.17	
Total	108	287.33			87	223.12		

* Significant at 1 % level

The analysis of variance of weekly gain in weight of rats of the second generation at the Indian Veterinary Research Institute, Izatnagar, is given in table 13.

The five fats differ significantly from each other, the highest rate of weight increase being recorded for ghee. If figures for ghee are excluded from analysis, the remaining fats are also found to differ significantly from each other, the growth rate being the lowest in the case of vanaspati, m. p., 41°C.

The analysis of variance of weekly gain in live weight of rats of the third generation at the Indian Veterinary Research Institute, Izatnagar, is given in table 14.

In the third generation, rats receiving ghee show a significantly higher rate of growth in comparison with the remaining fats. The other four fats also are found to differ significantly among themselves, the value being the highest for vanaspati, m. p., 37°C.

The analysis of variance of weekly gain in live weight of rats of the second generation at the Indian Institute of Science, Bangalore, is given in table 15.

Since the covariance between gain in weight and initial weight is significant, the within groups regression coefficient being 1.844 with a standard error of 0.29, the mean rates of growth were accordingly adjusted for initial weight and the adjusted figures are shown in Table 16.

Table 16 : Adjusted mean rates of weekly gain in live weight

	Ghee	Raw groundnut oil	Refined groundnut oil	Vaaspati, m.p., 37°C.	Vanaspati, m.p., 41°C.	All fats
			Males			
Sr. I	11.4	10.7	11.4	11.5	10.7	11.1
Sr. IV (a)	9.0	8.8	8.2	8.1	7.9	8.4
All diets	10.2	9.8	9.8	9.8	9.3	9.8
			Females			
Sr. I	7.6	6.3	8.2	8.7	8.0	7.8
Sr. IV (a)	6.4	5.3	6.3	7.3	7.3	6.5
All diets	7.0	5.8	7.2	8.0	7.6	7.3

The fats show significantly different rates of increase in weight, the highest rate being recorded for vanaspati, m. p., 37°C. For the male rats the lowest value is recorded for vanaspati, m. p., 41°C. and for the female rats for raw groundnut oil.

The differences between the remaining four fats after exclusion of ghee are statistically significant.

The analysis of variance of weekly gain in live weight of rats of the third generation at the Indian Institute of Science, Bangalore, is given in table 17.

Table 17: Analysis of variance of weekly gain in live weight of rats of third generation at the Indian Institute of Science, Bangalore

Source of variation	Including ghee				Excluding ghee			
	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Degrees of freedom	Sum of squares	Mean squares	F(observed)
Fats (F)	4	9.35	2.338	13.22*	3	6.930	2.310	13.51*
Sexes (S)	1	122.77	122.770	693.62*	1	121.580	121.580	710.99*
Diets (D)	1	584.93	584.930	3304.69*	1	505.540	505.540	2956.37*
Sex × fat	4	8.28	2.070	11.69*	3	1.925	0.642	3.75
Fat × diet	4	14.87	3.717	21.02*	3	11.840	3.947	23.08
Sev × diet	1	1.34	1.340	7.57*	1	0.751	0.751	4.39
F × S × D	4	1.94	0.485	2.74	3	1.783	0.594	3.47
Error	99	17.51	0.177		79	13.492	0.171	
Total	118				94	663.840		

* Significant at 1 % level

Since the covariance between gain in live weight and initial weight is significant, the within group regression coefficiant being 2.457 with a standard error of 0.44, the mean rates of increase were duly adjusted and the figures are presented in Table 18.

Table 18: Adjusted rates of weekly gain in live weight

	Ghee	Raw groundnut oil	Refined groundnut oil	Vanaspati, m.p., 37°C.	Vanaspati, m.p., 41°C.	All fats
Males						
Sr. I	11.5	13.4	13.3	12.9	11.6	12.5
Sr. IV (a)	7.4	7.3	8.2	5.6	8.1	7.9
All diets	9.5	10.4	10.8	9.3	9.9	10.2
Females						
Sr. I	10.0	10.7	10.2	10.6	10.0	10.3
Sr. IV (a)	6.7	5.5	6.0	6.4	6.0	6.1
All diets	8.4	8.1	8.1	8.5	8.0	8.2

The 5 fats differ significantly in respect of growth rates, the highest rate being recorded for vanaspati, m.p., 37°C. If ghee is excluded from the analysis, the remaining fats also differ significantly.

C.—REPRODUCTIVE ABILITY OF RATS WHEN FED ON DIFFERENT EXPERIMENTAL FATS AND DIETS

The basic data for each group of female rats, kept on various combinations of diets and fats, relate to the numbers becoming pregnant in each group, the size of the litter and the total number of the young ones surviving the weaning period.

The reproductive capacity of female rats under different fats can be compared from the data given in Tables 19 and 20.

Table 19: Reproductive capacity of female rats

	Indian Dairy Research Institute, Bangalore		Indian Institute of Science, Bangalore		Indian Veterinary Res. Inst., Izatnagar		University Science College, Calcutta	
	Total female rats mated	Percentage of pregnant rats	Total female rats mated	Percentage of pregnant rats	Total female rats mated	Percentage of pregnant rats	Total female rats mated	Percentage of pregnant rats
Ghee	36	83.3	48	85.4	30	86.7	20	75.0
Raw groundnut oil	36	61.1	49	79.6	35	74.3	21	57.1
Refined groundnut oil	35	51.4	46	76.1	36	77.8	21	61.9
Vanaspati, m.p., 37°C.	36	58.3	46	82.6	36	80.6	21	57.1
Vanaspati, m.p., 41°C.	36	69.4	46	73.9	34	82.4	22	72.7
Total ..	179	64.8	235	79.6	171	80.1	105	64.8

Table 20 : All centres

	Total female rats mated	Percentage of pregnant rats
Ghee	134	83.6
Raw groundnut oil	141	70.2
Refined groundnut oil	138	68.1
Vanaspati, m.p., 37°C.	139	71.9
Vanaspati, m.p., 41°C.	138	74.6
Total ..	690	73.6

By means of the X2 test we can examine whether the proportion of pregnant rats differs for various fats.

The values of X2 for individual centres are as follows :

	X2	Significant
1. Indian Dairy Research Institute, Bangalore ..	9.38	Significant
2. Indian Institute of Science, Bangalore ..	2.52	Not significant
3. Indian Veterinary Research Institute, Izatnagar	1.79	do.
4. University Science College, Calcutta	2.67	do.

The value of X2 worked out on the total experience of all the four centres is 10.12 for 4 degrees of freedom. This value is statistically significant indicating that the fats differ in respect of their effect on reproductive capacity. It is clear from Table 20 that the percentage of pregnant rats is highest in the case of the ghee group. If this group is excluded, the value of X2 is found to be 1.54 which is not of statistical significance. It may be concluded, therefore, that in regard to reproductive capacity there is no statistically significant difference in vanaspati, or raw or refined groundnut oil.

Another method of comparing reproductive capacity would be to study the variation in the average size of litter of different groups of rats. Table 21 shows the average number of rats born per female rat in various diet and fat groups.

Table 21 : Average size of litter in different diet and fat groups

	Ghee	Raw groundnut oil	Refined groundnut oil	Vanaspati, m.p., 37°C.	Vanaspati, m.p., 41°C.	All fats
	Indian Dairy Research Institute, Bangalore					
Sr. I	4.5	3.7	0.0	4.2	5.5	3.6
Sr. II	4.0	2.3	2.4	3.0	2.7	2.9
Sr. III	6.8	3.7	5.5	5.2	5.8	5.4
Sr. IV	2.0	2.3	4.2	0.0	0.7	1.8
Sr. V	6.5	3.7	1.7	2.3	3.5	3.5
Sr. VI	6.5	3.0	5.7	6.3	5.5	5.4
All diets	5.1	3.1	3.3	3.5	4.0	3.8

Table 21—*Continued*

	Ghee	Raw groundnut oil	Refined groundnut oil	Vanaspati, m.p., 37°C.	Vanaspati. m.p.. 41°C.	All fats
Indian Institute of Science, Bangalore						
Sr. I	7.5	8.5	8.8	6.3	7.3	7.7
Sr. II	4.5	2.5	2.5	4.0	2.2	3.1
Sr. III	5.3	4.7	2.7	2.8	2.7	3.6
Sr. IV	3.3	3.3	3.0	1.9	2.4	2.8
Sr. IV(a)	4.7	3.7	4.0	4.5	3.7	4.1
Sr. IV(b)	7.7	7.5	8.7	8.2	9.7	8.4
Sr. V	5.6	7.3	6.0	6.8	7.0	6.5
Sr. VI	3.8	2.3	1.7	4.3	2.0	2.8
All diets	5.3	5.0	4.7	4.9	4.6	4.9
Indian Veterinary Research Institute, Izatnagar						
Sr. I	6.8	4.6	4.2	6.0	5.4	5.4
Sr. II	6.3	4.0	5.8	6.7	4.0	5.4
Sr. III	7.0	6.5	7.3	8.0	8.0	7.4
Sr. IV	6.3	4.3	4.8	5.3	6.2	5.4
Sr. V	5.0	5.5	6.8	4.5	4.2	5.2
Sr. V(a)	8.0	6.0	7.8	4.7	6.3	6.6
All diets	6.6	5.2	6.1	5.9	5.7	5.9
University Science College, Calcutta						
Sr. II	3.7	1.3	0.0	2.3	1.0	1.7
Sr. III	2.0	4.2	5.5	6.8	5.8	4.9
Sr. IV	5.8	2.2	3.2	2.2	3.0	3.3
Sr. VI	2.8	4.3	5.8	3.3	5.0	4.2
All diets	3.6	3.0	3.6	3.7	3.7	3.5

The average size of litter is the largest for the ghee group. The figures of Table 21 were analysed by the technique of analysis of variance to test whether this difference is statistically significant or not. Two separate analyses were done, one on the figures of the first three centres with the five series of common diets (Table 22) and the other on all the four centres, each centre contributing only three diet groups (Table 23).

Table 22: Analysis of variance of average size of litter in three centres with five diets

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F observed
Fats	4	11.495	2.8738	1.92
Diets	4	62.204	15.5510	10.41*
Centres	2	66.103	33.0515	22.12*
Fat × diet	16	16.748	1.0468	<1
Fat × centre	8	10.889	1.3611	<1
Diet × centre	8	85.516	10.6895	7.15*
Error	32	47.812	1.4941	
Total	74	300.767		

* Statistically significant at 1 % probability level of significance.

Table 23: Analysis of variance of average size of litter in four centres with three diet groups

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)
Fats	4	11.542	2.886	2.10
Diets	2	54.711	27.326	19.90*
Centres	3	85.823	28.608	20.81*
Fat × diet	8	15.666	1.958	1.42
Fat × centre	12	10.998	0.916	< 1
Diet × centre	6	19.466	3.244	2.36
Error	24	32.990	1.375	
Total	59	231.196		

* Statistically significant at 1 % probaility level of significance

In none of these analyses the differences in average size of litter between the five fat groups emerge into statistical significance.

D.—LACTATING CAPACITY OF FEMALE RATS.

Lactating capacity has been measured by the ratio of the number of new born rats surviving 20 days to the total number of fertile mother rats. The experiments were carried out with different diets and with five fat supplements, viz. (1) raw groundnut oil, (2) refined groundnut oil (3) vanaspati, m.p., 37°C. (4) vanaspati, m. p., 41°C. and (5) ghee. Comparable data for five diets are available for only three centres, viz. the Indian Dairy Research Institute, Bangalore, Indian Institute of Science, Bangalore, and the Veterinary Research Institute, Izatnagar. Lactating capacity of rats under different diet and fat groups is shown in Table 24.

Table 24: Lactating capacity for different fat and diet groups

Diets	Ghee	Raw groundnut oil	Refined groundnut oil	Vanaspati, m.p., 37°C.	Vanaspati, m.p., 41°C.
Indian Dairy Research Institute, Bangalore					
I	0.8	2.0	0.0	0.6	1.5
II	0.0	0.0	0.0	0.0	2.0
III	3.5	1.8	2.6	3.5	2.5
IV	2.3	1.0	0.0	0.0	1.0
V	0.0	1.0	0.0	1.3	2.5
Indian Institute of Science, Bangalore					
I.	1.7	1.7	1.8	1.7	1.7
II	0.8	0.0	0.0	0.0	0.0
III	0.0	0.0	0.0	0.0	0.0
IV	0.0	0.0	0.0	0.0	0.0
V	0.0	0.5	0.0	0.5	0.5
Veterinary Research Institute, Izatnagar					
I	5.7	6.7	7.0	5.3	5.3
II	5.8	5.8	6.6	6.0	6.0
III	6.0	4.2	6.2	5.8	7.0
IV	6.0	4.0	7.3	4.4	6.6
V	6.0	7.0	7.6	6.0	5.3

The results of the analysis of variance on the above figures are set out in Table 25.

Table 25: Analysis of variance on lactating capacity

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the the probability level of: 5 %	1 %
Fat	4	2.030	0.5075	0.876	2.67	3.97
Diet	4	7.362	1.8405	3.177	2.67	3.97
Centre	2	452.357	226.1785	390.434	3.30	5.34
Interaction Diet × fat ..	16	8.606	0.5379	0.929	1.97	2.62
Interaction Fat × centre ..	8	9.711	1.2139	2.095	2.25	3.12
Interaction Diet × centre ..	8	21.475	2.6844	4.634	2.25	3.12
Error Diet × fat × centre	32	18.537	0.5793			

As is evident from Table 25 the fats do not differ in regard to lactating capacity. A similar analysis was carried out on data relating to synthetic diet from the three centres. The results of the analysis of variance are given in Table 26.

Table 26: Analysis of variance of lactating capacity for synthetic diet only

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of: 5%	1 %
Fats	4	1.467	0.367	0.917	3.84	7.01
Centres	2	73.444	36.722	82.763	4.46	8.65
Error	8	3.549	0.444			
Total ..	14					

The fats do not differ among themselves significantly. Similar analysis was carried out only with the figures relating to the Veterinary Research Institute, Izatngar. The results are shown in Table 27.

Table 27: Analysis of variance of lactating capacity for Veterinary Research Institute

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of: 5 %	1 %
Fats	4	6.778	1.694	2.307	3.01	4.44
Diets	4	1.438	0.359	0.486	3.01	4.44
Error	16	11.758	0.734			
Total ..	24					

The differences among the fats in respect of the lactating capacity are not significant.

E.—COMPARATIVE INFLUENCE OF DIFFERENT FATS IN RESPECT OF THE FAT CONTENT OF LIVERS, ASH CONTENTS OF FEMUR BONES AND VITAMIN A CONTENTS OF LIVERS

After the experiments on growth of rats had been continued for 12 weeks, male and female rats were mated by putting one male and one female rat in a cage for a period of one month. Thereafter the male rats were killed to determine the fat and vitamin A content of liver, and ash content of femur bones. The female rats were sacrificed two months after parturition for this purpose. The relevant data for three generations are available only from the Indian Institute of Science, Bangalore, in respect of two diets, viz. synthetic diet and poor Bengali diet. From the University College of Science and Technology, Calcutta, data in respect of the five fats are available for only one generation and for two diets (synthetic diet and poor Bengali diet). From the Indian Dairy Research Institute, Bangalore, only the average values for fat and vitamin A content of liver are available for one generation and for two diets, viz. synthetic and poor Bengali diets. The statistical analysis on these figures is shown separately for each centre.

(1) Indian Dairy Research Institute, Bangalore

The average percentage values of fat in liver are given in Table 28 for the first generation of rats separately for synthetic and poor Bengali diets.

Table 28: Average percentage of fat in liver for first generation rats

	Synthetic diet		Poor Bengali diet	
	Male	Female	Male	Female
Ghee	16.2	15.6	17.3	16.3
Raw groundnut oil	15.5	15.2	16.6	17.1
Refined groundnut oil ..	15.9	16.6	16.9	16.7
Vanaspati, m.p., 37°C. ..	16.2	15.8	17.2	16.6
Vanaspati, m.p., 41°C. ..	16.7	16.2	16.8	17.2

The results of statistical analysis are summarised in Table 29.

Table 29: Analysis of variance of percentage of fat in liver for first generation rats

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of: 5 %	1 %
Fat	4	0.8470	0.2118	1.382	6.39	15.98
Diet	1	3.8720	3.8720	25.266	7.71	21.20
Sex	1	0.2000	0.2000	1.305	7.71	21.20
Interaction						
Fat × diet	4	0.6030	0.1508	<1	6.39	15.98
Fat × sex	4	0.7650	0.1913	1.248	6.39	15.98
Diet × sex	1	0.0020	0.0020	<1	7.71	21.20
Error	4	0.6130	0.1533			
Total ..	19					

The fats do not show any statistically significant difference among themselves in respect of the first generation rats. The analysis is not based on individual observations of rats (which are not available) but only on average values.

Vitamin A content of liver—The average values of vitamin A in blue units per g. of fresh liver are given in Table 30 for one generation of rats under synthetic and poor Bengali diets.

Table 30: Vitamin A deposition in liver in respect of the first generation rats in blue units

	Synthetic diet		Poor Bengali diet	
	Male	Female	Male	Female
Ghee	0.72	0.68	1.10	1.20
Raw groundnut oil	0.64	0.73	0.98	0.82
Refined groundnut oil ..	0.68	0.72	1.20	1.30
Vanaspati, m. p., 37°C. ..	0.70	0.64	0.84	0.94
Vanaspati, m. p., 41°C. ..	0.72	0.68	0.91	0.91

The results of statistical anaylsis are shown in Table 31.

Table 31: Analysis of variance of vitamin A deposition in the liver of rats of first generation

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of: 5%	1%
Fat	4	0.1253	0.0313	4.641	6.39	15.98
Sex	1	0.0009	0.0009	<1	7.71	21.20
Diet	1	0.5412	0.5412	80.178	7.71	21.20
Interaction						
Fat × diet	4	0.1026	0.0257	3.800	6.39	15.98
Fat × sex	4	0.0070	0.0018	<1	6.39	15.98
Sex × diet	1	0.0011	0.0011	<1	7.71	21.30
Error	4	0.0270	0.0068			
Total ..	19					

The fats do not show any statistically significant difference among themselves in respect of vitamin A deposition in liver for the first generation.

For this centre, the analysis was not carried out on figures of rats of the second generation as the number of rats was very small.

(2) Indian Institute of Science, Bangalore

Table 32 gives the average values of percentages of fat in liver per rat for three consecutive generations of rats.

Table 32: Average percentage of fat in liver for five different fats in synthetic and poor Bengali diets for three consecutive generations

	Ghee	Raw groundnut oil	Refined groundnut oil	Vanaspati, m.p., 37°C.	Vanaspati, m.p., 41°C.
First generation					
Synthetic diet					
Male	18.20	18.20	18.80	18.20	18.67
Female	16.40	18.28	18.00	15.20	16.70
Poor Bengali diet					
Male	18.00	18.20	18.00	18.90	19.00
Female	17.97	17.40	17.00	18.10	18.30
Total	70.57	72.08	71.80	70.40	72.67
Mean	17.64	18.02	17.95	17.60	18.17
Second generation					
Synthetic diet					
Male	19.02	18.90	18.22	18.43	18.27
Female	17.60	17.90	18.28	17.45	17.00
Poor Bengali diet					
Male	18.62	18.42	19.20	19.32	19.88
Female	17.58	17.48	16.93	17.75	18.68
Total	72.82	72.70	72.63	72.95	73.83
Mean	18.21	18.18	18.16	18.24	18.46
Third generation					
Synthetic diet					
Male	17.92	18.17	18.40	18.57	17.87
Female	17.00	16.80	16.88	16.97	17.17
Poor Bengali diet					
Male	18.17	18.60	18.43	18.95	18.92
Female	17.32	17.03	18.03	19.10	18.87
Total	70.41	71.60	71.91	73.59	72.83
Mean	17.60	17.90	17.98	18.40	18.21

In the first and second generations vanaspati, m.p., 41°C. shows the highest degree of fat deposition while in the third generation the highest value has been recorded for vanaspati, m.p., 37°C.

The results of the analyses of variance are summarised in Table 33 separately for each generation.

Table 33: Analysis of variance of percentage of fat in liver separately for the three generations

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed	Value of F at the probability level of: 5%	1%
First generation						
Fat	4	5.8087	1.4522	6.525	2.46	3.51
Sex	1	35.1001	35.1001	157.718	3.94	6.90
Diet	1	5.3341	5.3341	23.968	3.94	6.90
Fat × sex	4	7.8986	1.9747	8.873	2.46	3.51
Fat × diet	4	28.5446	7.1362	32.065	2.46	3.51
Sex × diet	1	5.1667	5.1667	23.216	3.94	6.90
Fat × sex × diet	4	10.4121	2.6030	11.696	2.46	3.51
Error	100	22.2550	0.2226			
Total	119					
Second generation						
Fat	4	1.4603	0.3651	2.243	2.46	3.51
Sex	1	41.1841	41.1841	253.123	3.94	6.90
Diet	1	2.5521	2.5521	15.628	3.94	6.90
Fat × sex	4	0.4797	0.1199	0.737	2.46	3.51
Fat × diet	4	17.9950	4.4988	27.651	2.46	3.51
Sex × diet	1	1.5640	1.5640	9.611	3.94	6.90
Fat × sex × diet	4	7.1631	1.7908	11.007	2.46	3.51
Error	100	16.2716	0.1627			
Total	119					
Third generation						
Fat	4	8.9278	2.2320	15.43	2.46	3.51
Sex	1	18.3301	18.3301	126.75	3.94	6.90
Diet	1	22.6201	22.6201	156.41	3.94	6.90
Fat × sex	4	1.4662	0.3666	2.54	2.46	3.51
Fat × diet	4	4.9728	1.2432	8.60	2.46	3.51
Sex × diet	1	5.7641	5.7641	38.86	3.94	6.90
Fat × sex × diet	4	2.3004	0.5750	3.98	2.46	3.51
Error	100	14.4618	0.1446			
Total	119					

The differences in the deposition of fat in liver for the five different fats, i.e., ghee, raw groundnut oil, refined groundnut oil, vanaspati, m. p., 37°C. and vanaspati, m. p., 41°C. are found to be statistically significant in the first and third generations. For the second generation this difference is converging to significance at the 5 per cent level of F. The deposition of fat in liver in the case of vanaspati, m. p., 41°C. is significantly higher than that in the case of ghee in all the three generations. The deposition

due to vanaspati, m. p., 41°C. is also higher than those due to raw and refined groundnut oils in the second and third generations. Vanaspati, m. p., 37°C. does not differ in fat deposition in liver from ghee or raw or refined groundnut oil in the first and second generations. In the third generation both types of vanaspati (m. p., 37°C. and m. p., 41°C.) show high figures of fat deposition, that of vanaspati, m. p., 37°C. being the highest.

Ash content of femur bones—Table 34 give the averages percentage values of ash content in femur bones per rat for the three consecutive generations separatelty for the synthetic and poor Bengali diets.

Table 34: Average percentage of ash content in femur bones separately for the three generations

	Ghee	Raw groundnut oil	Refined groundnut oil	Vanaspati, m. p., 37°C.	Vanaspati, m. p., 41°C.
First generation					
		Synthetic diet			
Male	68.83	68.33	70.17	68.00	67.83
Female	70.83	69.17	68.17	68.67	69.67
		Poor Bengali diet			
Male	70.67	69.83	70.17	74.17	73.17
Female	72.00	74.17	72.83	70.33	67.67
Total ..	282.33	281.50	281.34	281.17	278.34
Mean ..	70.58	70.38	70.34	70.29	69.59
Second generation					
		Synthetic diet			
Male	69.33	68.83	67.17	69.17	67.17
Female	67.33	67.83	68.50	67.67	69.17
		Poor Bengali diet			
Male	70.17	71.00	70.83	70.67	69.67
Female	70.17	74.00	71.83	71.33	70.83
Total ..	277.00	281.66	278.33	278.84	276.84
Mean ..	69.25	70.42	69.58	69.71	69.21
Third generation					
		Synthetic diet			
Male	67.83	69.17	69.33	70.00	69.50
Female	64.17	65.00	64.50	64.33	63.83
		Poor Bengali diet			
Male	70.33	72.00	70.00	69.00	69.83
Female	70.67	73.50	73.00	71.50	71.17
Total ..	273.00	279.67	276.83	274.83	274.33
Mean ..	68.25	69.92	69.21	68.71	68.58

On the average the lowest ash content of femur bone has been recorded for vanaspati, m.p., 41°C. and the highest for raw groundnut oil.

For the purpose of assessing the relative differences of ash content in femur bones in the case of different fats, analysis of variance was carried out. Results are summarised in Table 35.

Table 35: Analysis of variance of percentages of ash content in femur bons for the three generations

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of. 5%	1%
First generation						
Fat	4	13.8834	3.4709	<1.000	2.46	3.51
Sex	1	1.6334	1.6334	<1.000	3.94	6.90
Diet	1	192.5334	192.5334	53.426	3.94	6.90
Fat × sex	4	90.9499	22.7375	3.949	2.46	3.51
Fat × diet	4	25.7166	6.4292	1.116	2.46	3.51
Sex × diet	1	5.6332	5.6332	<1.000	3.94	6.90
Fat × sex × diet	4	157.1168	39.2792	6.819	2.46	3.51
Error	100	576.0000	5.7600			
Total	119					
Second generation						
Fat	4	22.7834	5.6959	6.007	2.46	3.51
Sex	1	6.5334	6.5334	6.891	3.94	6.90
Diet	1	240.8334	240.8334	253.998	3.94	6.90
Fat × sex	4	29.1776	7.4292	7.835	2.46	3.51
Fat × diet	4	23.0832	5.7708	6.068	2.46	3.51
Sex × diet	1	14.6999	14.6999	15.503	3.94	6.90
Fat × sex × diet	4	23.4001	5.8500	6.170	2.46	3.51
Error	100	94.8167	0.9482		..	
Total	119					
Third generation						
Fat	4	40.3334	10.0959	18.280	2.46	3.51
Sex	1	70.5334	70.5334	127.708	3.94	6.90
Diet	1	563.3334	563.3334	1019.978	3.94	6.90
Fat × sex	4	5.0499	1.2625	2.286	2.46	3.51
Fat × diet	4	22.0832	5.5208	9.996	2.46	3.51
Sex × diet	1	320.1332	320.1332	579.636	3.94	6.90
Fat × sex × diet	4	16.7167	4.1792	7.567	2.46	3.51
Error	100	55.2335	0.5523			
Total	119					

The fats show significant differences in the second and third generations; the ash content of femur bones in the case of raw groundnut oil being the highest and being the lowest for vanaspati. m. p., 41°C.

Storage of vitamin A in liver in I. U.—Table 36 gives the vitamin A in liver in I. U. of rats for the three consecutive generations in respect of synthetic and poor Bengali diets.

Table 36: Vitamin A content of liver in I. U. of rats for the three generations

	Ghee	Raw groundnut oil	Refined groundnut oil	Vanaspati, m.p., 37 C.	Vanaspati, m.p., 41°C.
First generation					
		Synthetic diet			
Male	735	794	696	594	735
Female	833	852	1068	970	882
		Poor Bengali diet			
Male	1882	1548	1058	1597	1480
Female	1150	1654	970	852	852
Total ..	4600	4848	3792	4013	3949
Mean ..	1150.00	1212.00	948.00	1003.25	987.25
Second generation					
		Synthetic diet			
Male	853	823	853	860	863
Female	764	804	794	774	715
		Poor Bengali diet			
Male	1793	1588	1345	1656	1589
Female	1587	1597	1676	1637	1558
Total ..	4997	4812	4668	4927	4715
Mean ..	1249.25	1203.00	1167.00	1231.75	1178.75
Third generation					
		Synthetic diet			
Male	892	892	912	853	902
Female	1000	902	1078	1009	921
		Poor Bengali diet			
Male	1323	1401	1294	1274	1284
Female	1147	1107	1058	1186	1078
Total ..	4362	4302	4342	4222	4185
Mean ..	1090.50	1075.50	1085.50	1080.50	1046.25

The foregoing records of vitamin A content of liver were statistically analysed separately for each generation. The results are summarised in Table 37.

Table 37: Analysis of variance of vitamin A content of liver for the three generations

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of: 5%	1%
First generation						
Fats	4	209044.30	52261.08	1.058	6.39	15.98
Sex	1	53664.80	53664.80	1.086	7.71	21.20
Diet	1	1192672.80	1192672.80	24.135	7.71	21.20
Fat × sex	4	165592.70	41398.18	<1.000	6.39	15.98
Fat × diet	4	289471.70	72367.93	1.464	6.39	15.98
Sex × diet	1	492352.20	49235.20	9.963	7.71	21.20
Error	4	197665.30	49416.33			
Total	19					
Second generation						
Fat	4	19224.70	4806.18	<1.000	6.39	15.98
Sex	1	4712.50	4712.45	<1.000	7.71	21.20
Diet	1	3146624.50	3146624.50	366.460	7.71	21.20
Fat × sex	4	45461.30	11365.33	1.320	6.39	15.98
Fat × diet	4	20608.30	5152.08	<1.000	6.39	15.98
Sex × diet	1	11281.30	11281.30	1.310	7.71	21.20
Error	4	34346.50	8586.63			
Total	19					
Third generation						
Fat	4	4821.80	1205.45	1.010	6.39	15.98
Sex	1	14634.05	14634.05	12.260	7.71	21.20
Diet	1	389484.05	389484.05	326.260	7.71	21.20
Fat × sex	4	17809.20	4452.30	3.730	6.39	15.98
Fat × diet	4	16278.20	4069.55	3.410	6.39	15.98
Sex × diet	1	106434.05	106434.05	89.160	7.71	21.20
Error	4	4775.20	1193.80			
Total	19					

The fats do not show any significant difference in respect of vitamin A content of liver in any one of the three generations.

(3) University College of Science and Technology, Calcutta.

In Table 38 the values of percentage of fat in liver, dry weight basis, are given for each rat of the first generation, for the five different fats in respect of synthetic and poor Bengali diets.

Table 38 : Percentage of fat in liver of rats of the first generation

Rats	Ghee	Raw groundnut oil	Refined groundnut oil	Vanaspati, m. p., 37°C.	Vanaspati, m. p., 41°C.
			Synthetic diet		
1	9.7	4.7	4.0	5.8	6.4
2	10.7	5.5	6.4	11.1	11.2
3	9.6	10.1	6.0	12.0	11.2
4	13.0	10.1	6.0	8.3	8.1
5	5.9	8.6	6.2	11.1	7.5
6	9.0	8.4	10.9	8.5	9.3
Total	57.9	47.4	39.5	56.8	53.7
			Poor Bengali diet		
1	8.8	4.5	5.3	5.7	6.7
2	10.1	5.5	4.1	8.1	8.7
3	9.1	6.6	8.1	9.7	13.2
4	6.5	6.9	4.9	7.5	8.8
5	5.7	9.4	6.8	7.9	5.9
6	5.5	6.3	8.6	9.1	8.1
Total	46.7	39.2	37.8	48.0	51.4
Mean (all diets)	8.72	7.22	6.44	8.73	8.76

The results of statistical analysis are summarised in Table 39.

Table 39 : Analysis of variance of percentage of fat in liver

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of: 5%	1%
Fat	4	55.9790	13.99475	3.263	2.56	3.72
Diet	1	17.2806	17.28060	4.029	4.03	7.17
Fat × diet	4	5.9110	1.47775	<1	2.56	3.72
Error	50	214.4467	4.28893			
Total ..	59					

The fats show a significant difference among themselves at the 5 per cent. probability level. Here also Vanaspati, m. p., 41°C. shows the greatest deposition of fat in liver, which is in conformity with the results obtained at the Indian Institute of Science, Bangalore.

Vitamin A content of liver.—The values of vitamin A in I. U. per g. of dry liver for each rat, for the five different fats are given in Table 40.

Table 40: Vitamin A content in I. U. per g. of liver

Rats	Ghee	Raw groundnut oil	Refined groundnut oil	Vanaspati, 37°C m.p.	Vanaspati, 41°C m. p.
			Synthetic diet		
1	180	404	238	318	212
2	176	400	316	310	220
3	125	291	297	352	149
4	178	212	259	239	213
5	207	372	159	279	245
6	192	383	238	307	199
Total	1058	2062	1507	1805	1238
			Poor Bengali diet		
1	477	202	109	180	413
2	477	121	85	106	485
3	212	218	136	106	191
4	424	209	142	156	120
5	148	207	200	142	106
6	127	211	198	139	117
Total	1865	1168	870	829	1432
Mean	243.58	269·17	198.08	219.50	222·50

The data given in Table 40 on statistical analysis gave results as shown in Table 41.

Table 41: Analysis of variance of vitamin A content of liver

	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of: 1%	5%
Fat	4	34824.23	8706.06	1.208	2·58	3·72
Diet	1	37800.60	37800.60	5.246	4·03	7·17
Fat × diet ..	4	199406.90	49851.73	6.918	2·56	3·72
Error	50	360310.00	7206.22			

The five fats do not show any statistically significant difference in respect of the vitamin A deposition in the liver of rats.

Section II.—Metabolism Studies on adult human subjects, children and rats

METABOLISM STUDIES ON ADULT HUMAN SUBJECTS

INDIAN INSTITUTE OF SCIENCE, BANGALORE.

Metabolism studies were undertaken on twelve adult human subjects. Each individual was given five different fats, viz., raw groundnut oil, refined groundnut oil, vanaspati, m. p., 37°C., vanaspati, m. p., 41°C. and ghee at five different periods.

In the case of the first two fats the experimental period lasted 17 days over which the fat was administered. Of this period, 7 days constituted the preliminary period over which the subjects could get used to the fat and the remaining 10 days were divided into two periods of 5 days each, over each of which separate observations of urinary and faecal excretion were recorded. In the case of vanaspati, m.p., 37°C., vanaspati, m. p., 41°C. and ghee, however, after the first seven days of adaptation period, observations were made in a single five-day period only.

Table 42: Average daily intake of food and faecal excretion of seven human subject for different fats

		Subject						
		3 R.N.L.	5 M.M.D.	6 K.N.B.	7 K.B.B.	8 A.M.C.	10 D.H.H.	12 K.S.S.
Raw Groundnut oil								
First Period of study	A	744	790	806	880	775	840	890
	B	25.4	47.7	46.1	47.2	38.6	54.2	72.4
First Period of study	A	740	786	812	880	782	848	890
	B	26.2	56.8	53.9	56.6	29.8	45.4	66.0
Refined Groundnut oil								
First period of study	A	748	792	820	890	782	845	882
	B	25.1	34.5	45.4	46.8	30.8	33.1	54.7
Second period of study	A	748	792	812	885	793	845	888
	B	26.2	41.8	55.8	36.9	32.3	49.6	49.0
Vanaspati, m. p., 37°C.								
	A	750	786	826	885	793	852	888
	B	32.0	49.9	69.7	46.5	29.2	52.5	86.1
Vanaspati, m. p., 41°C.								
	A	756	782	818	892	798	865	908
	B	38.4	47.8	55.6	42.5	57.9	54.0	85.5
Ghee								
	A	764	780	828	882	790	865	900
	B	37.2	32.4	54.8	37.5	30.3	35.3	75.6

A : Average daily intake of food (dry weight) g.

B : Average daily excretion of faeces (dry weight) g.

Pairs of observations available in the case of raw and refined groundnut oils are thus made up of first and second periods of study. Data are also available in respect of the intake of food in each case.

Five subjects could not join one series or the other or the second period of metabolism study, owing to reasons unconnected with the experiments, although complete data for these subjects are available with reference to the remaining series of experiments or the first period of metabolism study. Statistical analysis has, therefore, been carried out with reference to seven subjects only.

Table 42 shows the average daily intake of food and the total amount of faeces excreted by the seven individuals, during the first and second periods of study in the case of the raw and refined groundnut oils and the single observations in the case of the other three fats.

Variation in total faecal excretion—A preliminary examination was made to see whether the total faecal excretion was correlated with the food intake. By the technique of analysis of co-variance the regression co-efficient of faecal excretion on intake of food was worked out.

The within regression co-efficient of total faecal excretion on food intake was not found to be statistically significant, indicating that the daily intake of food did not, on the whole, affect the total excretion. No allowance, therefore, seemed necessary for the variation in the daily intake of food in the subsequent analysis of total faecal excretion.

The significance of the differences in faecal excretion produced by different fats has been tested by the analysis of variance and is summarised below in Table 43.

Table 43: Analysis of variance of the total faecal excretion

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of: 5%	1%
Fats	4	1372.048	343.012	4.62	2.78	**3.22**
Subjects	6	7088.349	1181.392	15.90	2.51	**3.67**
Fat × subject	24	1783.708	74.321			
Error	14	498.075	35.577			
Total ..	48					

The conclusion drawn from Table 43 is that total excretion did vary significantly for different fats. Vanaspati, m. p., 41°C. produced the largest amount of faecal excretion ; the next in order being vanaspati, m. p., 37°C. The average daily dry weight of faecal excretion in respect of each fat is as follows :

Vanaspati, m. p., 41°C.	54.53 g.
Vanaspati, m. p., 37°C.	52.27 g.
Raw groundnut oil	47.59 g.
Ghee	43.30 g.
Refined groundnut oil	40.14 g.

Variation in the absorption of supplemented fat—In Table 44 are set out figures of percentage absorption of supplemented fats. These were calculated from the differences between the amounts of fat ingested and the faecal fat minus the fat excretion on practically fat free diet.

Table 44: Percentage absorption of supplemented fats

Subject No.	Raw groundnut oil		Refined groundnut oil		Vanaspati, m. p., 37°C.	Vanaspati, m. p., 41°C.	Ghee	Total
	First period of metabolism study	Second period of metabolism study	First period of metabolism study	Second period of metabolism study				
3	92.62	92.28	91.90	91.62	90.84	87.40	90.14	636.80
5	95.60	95.48	97.10	96.84	93.94	93.02	95.06	667.04
6	94.82	94.44	95.30	94.94	92.36	88.22	92.84	652.92
7	96.20	96.56	98.02	97.62	95.24	91.26	96.64	671.54
8	93.10	93.54	93.90	94.36	91.54	86.22	92.02	644.68
10	97.32	96.90	98.82	99.14	95.60	92.10	95.72	675.60
12	97.02	97.32	99.20	98.94	95.42	92.76	96.14	676.80
Total ..	1333.20		1347.70		654.94	630.98	658.56	4625.38
Mean ..	95.229		96.264		93.563	90.140	94.080	

It is apparent from Table 44 that for each of the seven human subjects vanaspati, m. p., 41°C., shows the lowest percentage of fat absorption, the next in order being vanaspati, m. p., 37°C. The significance of these low percentages is tested by the analysis of variance.

Table 45: Analysis of variance of percentage absorption of fat

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of: 5%	1%
Fats	4	190.965	47.739	65.85	2.78	4.22
Subjects	6	218.213	36.369	50.16	2.51	3.67
Fat × subject.	24	17.404	7.0725			
Error	14	0.841	0.060			
Total ..	48					

Table 45 reveals that the percentage absorption of fats is statistically different for different fats. The vanaspati, m. p., 41°C. shows the lowest percentage of absorption of 90.140 per cent. Also vanaspati, m.p., 37°C. shows a low percentage of absorption of 93.563 compared with 95.229 per cent., 96.264 per cent., and 94.080 per cent. for raw groundnut oil, refined groundnut oil and ghee respectively.

Absorption of phosphorus, calcium and protein—In this experiment, data were also recorded in respect of the relative effects of raw and refined groundnut oils, vanaspati m.p., 37°C. vanaspati, m.p., 41°C. and ghee on phosphorus, calcium and protein metabolism of human subjects.

In each case the balance absorption was worked out from the difference between the daily intake and the daily output (faecal as well as through urine).

Relative effects of various fats on phosphorus metabolism of human subject are given in Table 46.

Table 46: Daily absorption of phosphorus (in g.)

Subject No.	Raw groundnut oil		Refined groundnut oil		Vanaspati, m. p., 37°C.	Vanaspati, m. p., 41°C.	Ghee	Total	Mean
	1st period of metabolism study	2nd period of metabolism study	First period of metabolism study	Second period of metabolism study					
3	0.201	0.229	0.147	0.189	0.218	0.205	0.197	1.386	0.198
5	0.096	1.170	0.215	0.199	0.203	0.152	0.169	1.204	0.172
6	0.310	0.182	0.219	0.276	0.289	0.293	0.341	1.910	0.273
7	0.094	0.139	0.192	0.210	0.225	0.133	0.150	1.143	0.163
8	0.110	0.158	0.169	0.139	0.178	0.122	0.110	0.986	0.141
10	0.139	0.150	0.192	0.206	0.183	0.198	0.281	1.349	0.193
12	0.158	0.180	0.191	0.219	0.208	0.214	0.249	1.419	0.203
Total	2.316		2.763		1.504	1.317	1.497	9.397	
Mean	0.165		0.197		0.215	0.188	0.214		

Table 46 would seem to suggest that the absorption of phosphorus was the highest in the case of vanaspati, m. p., 37°C. By means of analysis of variance, given in Table 47, we may test whether the fats show any significant difference.

Table 47: Analysis of variance of phosphorus absorption

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of: 5%	1%
Fats	4	0.01739	0.00435	3.18	2.78	4.22
Subjects	6	0.07370	0.01228	8.96	2.51	3.67
Fat × subject	24	0.03282	0.00137			
Error	14	0.01753	0.00125			
Total ..	48					

In Table 47 the difference in the absorption of phosphorus with different fats is significant at the 5 per cent. probability level but not at 1 per cent. level.

The absorption of phosphorus is least in the case of raw groundnut oil and highest for vanaspati, m. p., 37°C. It may be stated that although the metabolism experiment at Coonoor yielded the lowest figure for phosphorus absorption for vanaspati, m. p., 41°C. the corresponding figure for vanaspati, m. p., 37°C. was quite high being exceeded only by that of ghee.

Relative effects of various fats on calcium metabolism are given in Table 48.

Table 48: Daily absorption of calcium (in g.)

Subject No.	Raw groundnut oil		Refined groundnut oil		Vanaspati, m. p., 37°C.	Vanaspati, m. p., 41°C.	Ghee	Total	Mean
	1st period of metabolism study	2nd period of metabolism study	1st period of metabolism study	2nd period of metabolism study					
3	0.091	0.118	0.104	0.127	0.112	0.136	0.123	0.811	0.116
5	0.099	0.043	0.109	—0.004	0.131	0.119	0.139	0.636	0.091
6	0.143	0.171	0.081	0.105	0.133	0.051	—0.002	0.682	0.097
7	0.192	0.148	0.127	—0.002	0.108	0.073	0.116	0.762	0.109
8	0.009	0.110	0.129	0.092	0.079	—0.003	0.082	0.498	0.071
10	—0.008	0.049	0.061	0.097	0.033	0.112	0.125	0.469	0.067
12	—0.006	0.012	0.140	0.129	0.173	0.153	0.159	0.760	0.109
Total	1.171		1.295		0.769	0.641	0.742	4.618	
Mean	0.084		0.093		0.110	0.092	0.106		

The results of variance of calcium absorption are given in Table 49.

Table 49: Analysis of variance of calcium absorption

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Fats	4	0.00434	0.00108
Subjects	6	0.01528	0.00255
Fat × subject	24	0.09091	0.00379
Error	14	0.02683	0.00192
Total ..	48		

The fats do not show significant differences among themselves.

Relative effects of various fats on protein metabolism are given in Table 50.

Table 50: Daily absorption of protein (in g.)

Subject No.	Raw groundnut oil		Refined groundnut oil		Vanaspati, m. p., 37°C.	Vanaspati, m. p., 41°C.	Ghee	Total	Mean
	1st period of metabolism study	2nd period of metabolism study	1st period of metabolism study	2nd period of metabolism study					
3	2.182	2.229	2.099	2.340	2.295	2.073	2.196	15.414	2.202
5	3.059	3.521	2.919	3.270	3.410	3.108	2.993	22.280	3.182
6	2.870	3.118	2.956	3.290	3.210	2.751	3.093	21.288	3.041
7	3.420	3.508	3.624	3.410	3.601	3.312	3.397	24.281	3.469
8	2.220	2.615	2.502	2.321	2.541	2.300	2.241	16.740	2.391
10	3.290	3.401	3.280	3.194	3.523	3.004	3.125	22.817	3.260
12	3.444	3.701	3.692	3.840	3.758	3.551	3.612	25.598	3.657
Total	42.587		42.737		22.338	20.099	20.657	148.418	
Mean	3.042		3.053		3.191	2.871	2.951		

The results of the analysis of variance of protein absorptions are given in Table 51.

Table 51: Analysis of variance of protein absorption

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of: 5%	1%
Fats	4	0.41088	0.10272	3.131	3.11	5.03
Subjects	6	12.28472	2.04745	62.417	2.85	4.46
Fat × subject	24	0.21763	0.00907	0.276	2.35	3.43
Error	14	0.45924	0.03280			
Total ..	48					

The fat groups show a significant difference at the 5 per cent. level only. The highest figure is shown by vanaspati, m. p., 37°C.

Analysis of Iodine numbers of plasma fatty acids—The Iodine numbers of plasma fatty acids of the subjects with reference to different fats have been compared in pairs. The results are summarised in Table 52. The comparisons are based on unequal numbers of observations because for some subjects the observations are not available Proper allowance for this inequality of numbers has been made.

Table 52: Comparison of iodine numbers of plasma fatty acids of subjects on different fats

Comparison between	Mean values and difference of Iodine numbers		Degrees of freedom	Standard error of the mean difference	't'	5% level of 't'	Statistical significance
Raw groundnut oil and Vanaspati, m.p., 37°C.	Vanaspati Raw Oil Difference	142.67 162.67 20.00	6	2.31	8.66	2.45	Significant
Refined groundnut oil and Vanaspati, m.p., 37°C.	Vanaspati Refined oil Difference	138.14 159.43 21.29	7	1.55	13.70	2.36	Significant
Raw groundnut oil and Refined groundnut oil	Raw oil Refined oil Difference	162.80 164.40 1.60	5	2.64	0.61	2.57	Not significant
Ghee and Vanaspati, m. p., 37°C.	Ghee Vanaspati Difference	137.83 141.50 3.67	5	2.12	1.73	2.57	Not significant.
Ghee and Vanaspati, m. p., 41°C.	Ghee Vanaspati Difference	139.4 140.6 1.2	4	2.75	0.44	2.78	Not significant
Vanaspati, m.p., 37°C. and Vanaspati, m.p., 41°C.	V., m.p., 37°C V., m.p., 41°C. Difference	142.83 140.33 2.50	5	1.89	1.32	2.57	Not significant
Ghee and Raw groundnut oil	Ghee Raw oil Difference	139.57 159.28 19.71	6	2.09	9.43	2.44	Significant
Ghee and Refined groundnut oil	Ghee Refined oil Difference	137.6 161.4 23.8	4	3.31	7.19	2.78	Significant
Refined groundnut oil and Vanaspati, m.p., 41°C.	Refined oil Vanaspati Difference	160.6 139.8 20.8	4	3.84	5.42	2.78	Significant

The iodine numbers of plasma fatty acids of subjects are high for raw and refined groundnut oils and low for ghee or vanaspati, m.p., 37°C., or m.p., 41°C. No difference is established between the iodine numbers for ghee or for vanaspati of either of the two melting points. Further, no difference is found to exist between raw and refined groundnut oils in respect of iodine numbers of plasma fatty acids. The values for both raw and refined groundnut oils are, however, found to be significantly higher than those for vanaspati, m. p., 37°C. or m., p., 41°C. or for ghee.

METABOLISM STUDIES ON CHILDREN BELOW THE AGE OF 11 YEARS

Indian Institute of Science, Bangalore.

This study was carried out by the Indian Institute of Science, Bangalore, in an orphanage, on two groups of six girls each; all the girls being below eleven years of age. Their weights ranged from 45 lbs. to 60 lbs. Girls were chosen mainly because they were more amenable to discipline, and it was presumed that growing children would show a better response than adults to additions in their dietary.

The experimental diet used, the so-called poor rice diet, was the one to which they had been accustomed to ever since their admission to the orphanage. The extent of retention and excretion of dietary fat, protein, calcium and phosphorus was studied. The diet of one group was supplemented with 4 per cent. to 6 per cent. of vanaspati and of the other groups with raw groundnut oil. Before actually collecting the excreta preliminary adaptation period of 10 days was allowed. Excreta were then collected for 72 hours.

In Table 53 are shown the percentages of fat absorption separately for the six individuals of each group as well as the percentage of faecal split and unsplit fat.

Table 53: Percentage of fat absorption and percentages of split and unsplit faecal fat

Subjects	Absorption of fat%	Faecal fat	
		Split %	Unsplit %
Raw Groundnut oil			
1. AA	82.5	81.4	18.6
2. PL	87.6	78.3	21.7
3. M	88.0	79.2	20.8
4. AP	81.4	83.1	16.9
5. PG	88.6	76.5	23.5
6. CP	83.6	81.6	18.4
Vanaspati, m.p., 37°C.			
1. PR	52.2	78.6	21.4
2. L	78.2	73.9	26.1
3. MPP	80.1	71.4	28.6
4. LRM	68.0	70.2	29.8
5. LMK	73.8	80.9	19.1
6. M	76.5	79.2	28.3

From the statistical point of view it is difficult to judge whether the two groups, viz., oil and vanaspati were strictly comparable at the beginning of the experiment. However, in the first place, the ' t ' test carried out on the percentage of unsplit fat in the two groups failed to bring out any statistically significant difference. This result is helpful in as much as it would seem to indicate that the two groups may not be considered different at least in regard to split fat percentages. The relative degree of fat absorption in the two groups was tested. The mean percentage absorption for raw groundnut oil is 85.28 as against 76.47 in the case of vanaspati. The value of ' t ' for 10 degrees of freedom is 3.63 which is significant

at 1 per cent. probability level. The conclusion drawn, therefore, is that absorption of fat is significantly less in the case of vanaspati, m. p., 37°C. The result is in conformity with those observed in the metabolism studies on human adults carried out by the same Institute and at Coonoor.

The average values of calcium, phosphorus and protein retention for both the groups are shown in Table 54.

Table 54: Average retention values of calcium, phosphorus and nitrogen (in g.) for the two groups of children

Fat group	Subject No.	Nitrogen	Calcium	Phosphorus
Raw groundnut oil	1	0.215	86.8	513
	2	0.964	233.3	589
	3	0.896	31.6	614
	4	0.901	101.6	719
	5	0.168	116.1	499
	6	0.834	112.6	531
Vanaspati, m. p., 37°C. ..	1	0.945	135.6	734
	2	0.142	73.6	641
	3	0.330	26.8	751
	4	0.349	88.2	837
	5	0.347	—17.1	805
	6	0.371	87.6	855

Comparisons between the two groups were carried out by the 't' test and the results obtained are given in Table 55.

Table 55: Results of 't' tests carried out for comparison between the two groups in respect of nitrogen, calcium and phosphorus retentions

Comparison between Vanaspati and Raw Groundnut oil in respect of	Observed value of 't'	Values of 't' at the probability level of: 5%	1%	Remarks
(*a*) Nitrogen ..	1.89	2.23	3.17	Not significant
(*b*) Calcium ..	1.38	2.23	3.17	Not significant
(*c*) Phosphorus ..	4.06	2.23	3.17	Significant. A higher value is observed in the case of vanaspati.

The two fat-groups failed to show any difference in regard to calcium and nitrogen metabolism, but there is an indication that phosphorus absorption is greater in the case of vanaspati group.

INDIAN INSTITUTE OF SCIENCE, BANGALORE.

Twelve young rats from stock colony were weaned on the twelfth day and after being kept on stock diet for a week were divided into two groups of six animals each, three being males and three females. The diet of one group was supplemented with vanaspati and of the other with butter. They were under observation for twelve weeks. Estimates of calcium and phosphorus absorption and faecal excretion were made at three stages, viz., Stage I at three weeks, Stage II at six weeks and Stage III at nine weeks of experiment.

Analysis of data relating to faecal excretion—Table 56 shows for the three successive stages the mean intake of food per rat and the faecal excretion separately for the different fat and sex groups. The figures relate to 4 day period in each case.

Table 56: Mean intake of food (in g.) per rat and the faecal excretion for three successive stages for the different fat and sex groups

		Butter fat group		Vanaspati group	
		Food consumed	Faecal excretion	Food consumed	Faecal excretion
Stage I	Female ..	23.1333	1.8300	21.8000	1.8400
	Male ..	22.4000	1.7333	21·9333	1.9333
Stage II	Female ..	34.5000	2.5467	34.1667	2.9833
	Male ..	35.5000	2.7067	34.8333	3.2433
Stage III	Female ..	35.6667	2.2867	35.7000	2.5100
	Male ..	36.3333	2.6700	34.6667	3.3433

In order to test whether faecal excretion was correlated with the food consumption the within regression co-efficients were calculated separately for each stage of experiment. The values of within regression co-efficients together with their standard errors are given in Table 57.

Table 57: Within regression co-efficients, with their standard errors for the three stages of the experiment

	Within regression co efficient	Standard error of the within regression co-efficient	Statistical Significance
Stage I	—0.2184	0.193	Not significant
Stage II	0.1188	0.532	,,
Stage III	—0.2142	0.177	,,

None of the regressions being significant the difference between fats has been tested by straight-forward analysis of variance. The analysis has been done separately for each stage of experiment because the rats used in the three stages were the same, the results obtained are given in Table 58.

Table 58: Analysis of variance of the quantity of faecal excretion

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at probability level of: 5%	1%
Stage I						
Fat	1	0.03307	0.03307	0.1308	5.32	11.26
Sex	1	0.00001	0.00001	0.0000	5.32	11.26
Fat × sex	1	0.02708	0.02708	0.1071	5.32	11.26
Error	8	2.02293	0.25287			
Total ..	11					
Stage II						
Fat	1	0.71053	0.71053	2.138	5.32	11.26
Sex	1	0.13230	0.13230	0.398	5.32	11.26
Fat × sex	1	0.00750	0.00750	0.023	5.32	11.26
Error	8	2.65827	0.33228			
Total ..	11					
Stage III						
Fat	1	0.603008	0.603008	3.431	5.32	11.26
Sex	1	1.110208	1.110208	6.317	5.32	11.26
Fat × sex	1	0.151876	0.151876	0.086	5.32	11.26
Error	8	1.405930	0.175742			
Total ..	11					

No difference has been established in regard to the amount of faecal excretion in the case of the two fats in any one of the three stages of experiments.

Calcium metabolism.—Table 59 shows mean figures of absorption of calcium (intake—excretion). The figures relate to 4-day period in each case.

Table 59: Mean figures of absorption (in mg.) of calcium

		Butter fat group	Vanaspati group
Stage I	Female	90.8667	115.9333
	Male	89.4000	95.7333
Stage II	Female	230.4667	221.9667
	Male	239.9667	236.8333
Stage III	Female	245.6333	256.7000
	Male	244.3000	229.2000

The significance of the difference between butter fat and vanaspati in regard to calcium absorption has been examined by the analysis of variance, separately carried out on figures for each stage of experiment. Results are shown in Table 60.

Table 60: Analysis of variance of the quantity of calcium absorption

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at probability level of: 5%	1%
Stage I						
Fat	1	739.4700	739.4700	3.960	5.32	11.26
Sex	1	352.0834	352.0834	1.886	5.32	11.26
Fat × sex	1	263.2033	263.2033	1.410	5.32	11.26
Error	8	1493.7000	186.7125			
Total ..	11					
Stage II						
Fat	1	101.5009	101.5009	0.609	5.32	11.26
Sex	1	445.3009	445.3009	2.670	5.32	11.26
Fat × sex	1	21.6007	21.6007	0.130	5.32	11.26
Error	8	1334.0267	166.7533			
Total ..	11					
Stage III						
Fat	1	12.2009	12.2009	0.052	5.32	11.26
Sex	1	623.5192	623.5192	2.639	5.32	11.26
Fat × sex	1	513.5224	513.5224	2.174	5.32	11.26
Error	8	1889.8667	236.2333			
Total ..	11					

In no case has a significant difference been established between vanaspati and butter fat.

Phosphorus metabolism—Table 61 shows mean figures of absorption of phosphorus (intake—excretion). The figures relate to 4-day period in each case.

Table 61: Mean figures of absorption of phosphorus

		Butter fat group	Vanaspati group
Stage I	Female	46.7000	43.5333
	Male	35.6000	47.5333
Stage II	Female	80.7667	78.3333
	Male	88.7333	74.8333
Stage III	Female	87.7333	89.7000
	Male	89.9000	77.2333

The significance of the difference between butter fat and vanaspati in regard to phosphorus absorption has been examined by the analysis of variance, separately carried out on figures of each stage of experiment. The results are given in Table 62.

Table 62: Analysis of variance of the quantity of absorption of phosphorus

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at the probability level of: 5%	1%
Stage I						
Fat	1	57.6409	57.6409	0.120	5.32	11.26
Sex	1	37.8075	37.8075	0.079	5.32	11.26
Fat × sex	1	171.0075	171.0075	0.356	5.32	11.26
Error	8	3845.6133	480.7017			
Total	11					
Stage II						
Fat	1	200.0834	200.0834	0.729	5.32	11.26
Sex	1	14.9634	14.9634	0.055	5.32	11.26
Fat × sex	1	98.6132	98.6132	0.359	5.32	11.26
Error	8	2195.7467	274.4683			
Total	11					
Stage III						
Fat	1	12.2009	12.2009	0.090	5.32	11.26
Sex	1	215.9009	215.9009	1.590	5.32	11.26
Fat × sex	1	340.2674	340.2674	2.505	5.32	11.26
Error	8	1085.5600	135.8200			
Total	11					

In regard to phosphorus absorption, also, in no case a significant difference has been established between vanaspati and butter fat.

Protein metabolism—The digestion co-efficient and biological value were worked out for each rat. The figures are given in Tables 63 and 64.

Table 63: Digestion co-efficient (in percentage) for each rat

		Ghee	Raw groundnut oil	Vanaspati, m. p., 37°C.	Coconut oil
Female	1	100.0	91.4	94.2	99.6
	2	97.1	96.0	98.0	91.9
	3	93.3	88.0	88.7	92.9
Male	4	92.6	91.5	88.3	96.2
	5	90.1	83.7	92.4	83.1
	6	85.8	89.6	90.6	91.9

Table 64: Biological value (in percentage) for each rat

		Ghee	Raw groundnut oil	Vanaspati, m. p., 37°C.	Coconut oil
Female	1	77.1	79.2	73.3	79.1
	2	74.2	80.6	76.4	80.2
	3	78.6	75.8	81.5	82.5
Male	4	77.5	79.0	77.5	74.3
	5	72.7	75.8	75.9	78.8
	6	73.6	77.3	75.3	73.6

The rats used for the different fat groups being the same, the following analyses of variance were done, (*i*) with data for 3 female rats, (*ii*) with data for 3 male rats, (*iii*) with data for 6 rats irrespective of sex—. Table 65 summarises the results.

Table 65: Analysis of variance of figures of digestion co-efficient

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at probability level of: 5%	1%
			Female			
Fats	3	39.5625	13.1875	1.362	4.76	9.78
Female rats	2	75.5117	37.7559	31899	5.14	10.92
Fats × rats	6	58.0950	3.6825			
Total ..	11					
			Male			
Fats	3	9.3367	3.1122	0.173	4.76	9.78
Male rats	2	46.7450	23.3725	1.303	5.14	10.92
Fats × rats ..	6	107.6283	17.938			
Total ..	11					
			Male and Female			
Fats	3	33.2579	11.08587	0.917	3.29	5.42
Rats	5	249.6771	49.93542	4.130	2.90	4.56
Fats × rats	15	181.3646	12.09097			
Total ..	23					

In regard to digestion co-efficient, no significant difference has been established among the four fats, in any of the foregoing analyses.

Analysis of variance of figures of biological value of male and female rats is given in Table 66.

Table 66: Analysis of variance of figures of biological value of rats

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at probability level of: 5%	1%
			Female			
Fats	3	28.8292	9.60972	1.154	4.76	9.78
Female rats	2	12.5310	6.26550	0.752	5.14	10.92
Fats × rats	6	49.9690	8.32817			
Total ..	11					
			Male			
Fats	3	12.1692	4.0564	0.493	4.76	9.78
Male rats	2	9.1517	4.5759	0.562	5.14	10.92
Fats × rats	6	48.8292	8.1382			
Total ..	11					
			Male and female			
Fat	3	24.5383	8.1794	1.306	3.29	5.42
Rats	5	52.5100	10.5020	1.677	2.90	4.56
Fats × rats	15	93.9367	6.2624			
Total ..	23					

In regard to biological value also no significant difference has been established among the four fats.

To investigate whether 15 per cent. casein diet supplemented with vanaspati, m.p., 37°C., affected the absorption of calcium or phosphorus in comparison with the supplementation by other fats such as ghee, raw groundnut oil and coconut oil, an experiment with six rats, 3 females and 3 males, was carried out.

The rats were about 4 months old, female rats weighing about 150 g. each and the males about 200 g. each. The metabolism study lasted seven days, the first four days being considered as the initial acclamatisation period. Between each metabolism study, the animals were given a rest period of one week when they received the usual stock diet.

Table 67 gives the average daily absorption of phosphorus for males and females when the diet was supplemented with one or the other of the following four fats (1) ghee (2) vanaspati, m. p., 37°C. (3) raw groundnut oil and (4) coconut oil.

Table 67: Mean absorption of phosphorus (in mg.)

		Ghee	Groundnut oil	Vanaspati, m. p., 37°C.	Coconut oil
Female	1	3.5	15.3	28.3	6.8
	2	8.0	30.8	22.3	14.6
	3	−3.4	22.0	26.6	2.2
Sub-total		8.1	68.1	77.2	23.6
Male	4	5.4	17.3	13.3	14.7
	5	12.9	20.3	20.6	23.6
	6	7.6	21.3	20.6	12.2
Sub-total		25.9	58.9	54.5	50.5
Total		34.0	127.0	131.7	74.1

The results of the analysis of data relating to female rats are summarised in table 68.

Table 68: Analysis of variance of phosphorus absorption of female rats

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at probability level of: 5 %	1 %
Fat	3	1129.2567	376.4189	12.932	4 76	9.78
Rat	2	109.8650	54.9325	1.887	5.14	10.92
Interaction	6	174.6483	29.1081			

Table 68 shows that the absorption of phosphorus among female rats varies significantly from fat to fat, the absorption being the maximum in the case of vanaspati.

A similar analysis was carried out on the absorption figures of the male rats and the results are given in Table 69.

Table 69: Analysis of variance of phosphorus absorption of male rats

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at probability level of: 5 %	1 %
Fat	3	218.1700	72.7233	7.834	4.76	9.78
Rat	2	90.0317	45.0159	4.850	5.14	10.92
Interaction	6	55.6950	9.2825			

Here also the differences observed in the quantities of phosphorus absorbed are significant, the quantity absorbed in the case of raw groundnut oil being the highest followed by vanaspati.

Table 70 given the analysis on figures of all the six rats, irrespective of their sex.

Table 70: Analysis of variance of phosphorus absorption of male and female rats

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at probability level of: 5%	1%
Fat	3	1080.8566	360.2855	10.876	3.29	5.42
Rat	5	206.7233	41.3447	1.248	2.90	4.56
Interaction	15	496.9134	33.1276			

Here again the differences between fats are significant; the highest absorption being recorded in the case of vanaspati, m. p., 37°C.

Absorption of calcium—Table 71 gives the mean daily absorption of calcium for males and females when diet is supplemented with one or the other of the following fats: (1) ghee (2) vanaspati, m. p., 37°C. (3) raw groundnut oil and (4) coconut oil.

Table 71: Mean daily absorption of calcium (in mg.)

		Ghee	Groundnut oil	Vanaspati	Coconut oil
Female	1	1.2	0.8	8.2	2.8
	2	1.1	6.2	6.6	4.9
	3	0.5	8.6	3.5	3.1
	Sub-total	2.8	15.6	18.3	10.8
Male	4	0.7	3.5	4.1	9.8
	5	0.6	4.7	4.3	5.4
	6	0.3	9.3	8.2	7.0
	Sub-total	1.6	17.5	16.6	22.2
	Total	4.4	33.1	34.9	33.0

As in the analysis of data relating to phosphorus absorption, the observations on male and female rats were separately analysed and the results are shown in Table 72.

Table 72: Analysis of variance of calcium absorption of rats

Source of variation	Degrees of freedom	Sums of squares	Variance	F (observed)	Value of F at probability level of: 5%	1%
		Female				
Fat	3	46.2225	15.4076	2.201	4.76	9.78
Rat	2	4.2177	2.0189	0.301	5.14	10.92
Interaction	6	41.9890	6.9987			
Total ..	11					
		Male				
Fat	3	79.7025	26.5675	5.927	4.76	9.78
Rat	2	12.5450	6.2725	1.399	5.14	10.92
Interaction	6	26.8950	4.4825			
Total ..	11					

In the case of male rats the differences between the fats in the average quantities of calcium absorbed is significant, the quantity absorbed from ghee diet being the least. In the case of female rats, however, this difference is not significant.

Further an analysis on the quantities of calcium absorbed by all the six rats taken together, irrespective of their sex, showed that calcium absorption is significantly lower in the case of ghee than the corresponding quantities from other three fat diets. The results are shown in Table 73.

Table 73: Analysis of variance of clacium absorption of male and female rats

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at probability level of: 5%	1%
Fat	3	107.4483	35.8161	6.149	3.29	5.42
Rat	5	21.2633	4.2527	0.730	2.90	4.56
Interaction	15	87.3667	5.8244			
Total ..	23					

If the figures for ghee are excluded, the remaining three fats namely, groundnut oil, vanaspati and coconut oil do not show any statistical difference in their absorption. The analysis is summarised in Table 74.

Table 74: Analysis of variance of data relating to calcium metabolish for three fats, viz., vanaspati, m. p., 37°C., raw groundnut oil and coconut oil, of rats

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at probability level of: 5%	1%
		Male and female				
Fat	2	0.3811	0.1906	0.025	4.10	7.56
Rat	5	31.2578	6.2516	0.813	3.33	5.64
Interaction	10	76.8589	7.6859			
Total ..	17					
		Male				
Fat	2	6.0289	3.0145	0.563	6.94	18.00
Rat	2	17.9355	8.9678	1.675	6.94	18.00
Interaction	4	21.4178	5.3545			
Total ..	8					
		Female				
Fat	2	9.6200	4.8100	0.480	6.94	18.00
Rat	2	5.8433	2.9217	0.290	6.94	18.00
Interaction	4	40.0767	10.0192			
Total ..	8					

METABOLISM STUDIES ON ADULT HUMAN SUBJECTS

Nutrition Research Laboratories, Coonoor

Six healthy, young adult male workers in the Coonoor Laboratories who volunteered for study were each fed five different fats in succession; each fat being given for a period of 10 days. The order in which the five fats were administered was the same for all the six subjects. The quantity of fat fed per day was 68 g. per person. The first five days of each test-period were used for the purpose of allowing the subject to attain a constant metabolic condition in regard to the fat consumed and the second five-day period was used for the collection of excreta. Collection was made on the first two days, followed by a gap of one day and a second collection was made on the next two-day period. The total fat excreted during each two-day period was estimated.

Twenty-four hour samples of urine were collected and utilized for the estimation of calcium, phosphorus and nitrogen.

Table 75 shows the amount of fat excreted as percentage of the fat intake in the two two-day periods per person.

Table 75: Amount of fat excreted (in g.), as percentage of the fat intake by different human subjects when given the five different fats

Fat	Subjects						Total
	RMK	CS	NK	BK	TN	MT	
Vanaspati, m. p., 37°C. ..	4.185	6.809	8.460	5.276	4.989	8.101	75.916
	5.144	6.429	7.521	5.785	5.371	7.846	
Ghee	4.240	6.000	6.146	5.213	5.318	8.421	71.665
	4.865	5.953	7.358	4.795	4.765	8.591	
Vanaspati, m. p., 41°C. ..	12.893	6.460	11.581	9.247	6.346	11.661	121.463
	15.897	9.336	8.285	10.538	6.793	12.426	
Raw groundnut oil ..	4.980	6.051	7.140	4.236	5.903	8.764	71.506
	4.054	4.709	6.201	4.761	5.563	9.143	
Refined groundnut oil ..	5.254	5.226	6.865	4.937	5.726	8.844	76.784
	5.441	5.725	8.115	6.119	5.864	8.668	
Total	66.954	62.698	77.672	60.907	56.638	92.465	417.334

It is apparent from Table 75 that the excretion of fat was relatively greater in the case of vanaspati, m. p., 41°C. Excretion would also appear to differ in respect of the six human subjects. The significance of these variations has been tested by analysing the total variability of the 60 observations in the foregoing Table into that ascribed to:

(*i*) Variation in fats.

(*ii*) Variation in human subjects.

(*iii*) Interaction between fats and subject; this would be present if different subjects had a tendency to react differently to the fats.

(*iv*) Chance variation of which an estimate is obtained from the variability shown within each pair of observations on each person.

The results are summarised in the usual analysis of variance in Table 76.

Table 76: Analysis of variance of human metabolism data relating to fat excretion as percentage of total fat intake

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at the probability level of: 5%	1%
Fats	4	152.3107	38.0777	9.44	2.87	4.43
Human subjects ..	5	88.6174	17.7235	4.40	2.71	4.10
Fat × subject	20	80.6397	4.0320	5.66	1.93	2.56
Error	30	21.3868	0.7129			
Total ..	59	342.9546				

From this analysis we conclude that in respect of fat excretion :

(1) the five fats are significantly different. The highest excretion has occurred with vanaspati, m. p., 41°C.
(2) The subjects show a statistically significant tendency to react differently to different fats.

In order to test the significance of the high degree of excretion with vanaspati, m. p., 41°C., a similar analysis of variance was repeated on the data of Table 76 by excluding figures relating to this fat.

The analysis of variance is given in Table 77.

Table 77: Analysis of variance of fat excretion as percentage of total fat intake for vanaspati, m. p., 37°C., ghee, raw groundnut oil, and refined groundnut oil

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at the probability level of: 5 %	1 %
Fats	3	1.9242	0.6414	1.43	3.29	5.42
Human subjects ..	5	83.3267	16.6653	37.20	2·90	4.65
Fat × subject ..	15	6.7194	0.4480			
Error	24	6.0788	0.2532			
Total ..	47					

The variability between fats now ceases to possess statistical significance as also the interaction between subjects and fats.

The average amount of fat excreted in the case of each type of fat is shown in Table 78 together with the respective standard errors.

Table 78: Average amount of fat excreted in the case of each type of fat

Fat	Mean excretion (in g.)	Standard error
Vanaspati, m. p., 37°C	6.326	±0.669
Ghee	5.972	±0.669
Vanaspati, m. p., 41°C	10.122	±1.625
Raw groudnut oil	5.959	±0.669
Refined groundnut oil	6.399	±0.669

An illustration of the fact that different subjects reacted differently to vanaspati, m.p., 41°C. is provided by the subject R.M.K. who showed the highest excretion in respect of vanaspati, m. p., 41°C and the lowest excretion for the other four fats. This result is statistically significant in as much as by excluding this person from the analysis the interaction between subjects and fats ceases to be of statistical significance.

Similar analysis of variance was carried out on figures of excreted fat expressed as prcentages of total faeces. The percentage in respect of each subject and fat are shown in Tables 79 and 80.

Table 79: Excreted fat as percentage of faeces (dry weight) per period of two days, on a constant intake of 68 g. per day.

Subject	Vanaspati, m. p., 37°C	Ghee	Vanaspati, m. p., 41°C	Raw groundnut oil	Refined groundnut oil	Total
R M K	11.569	10.861	23.443	11.781	10.149	134.970
	9.909	10.854	24.765	10.810	10.819	
C B	9.439	10.570	17.819	12.959	15.218	140.022
	13.640	12.379	17.959	15.620	14.419	
N K	14.788	16.358	21.169	14.669	14.190	159.756
	16.209	14.419	20.120	14.055	13.779	
B K	12.040	14.440	18.440	11.230	11.210	142.632
	15.830	15.060	21.170	12.312	10.850	
T N	10.390	10.730	13.360	10.240	10.010	108.590
	9.130	12.560	11.050	11.310	9.810	
M T	16.950	14.970	22.180	15.480	14.960	167.890
	17.100	13.910	21.890	15.220	15.230	
Total ..	156.994	157.171	233.362	155.686	150.644	853.860
Mean ..	13.083	13.098	19.447	12.974	12.554	

Table 80: Analysis of variance of the records of the excreted fat as percentage of faeces (dry weight)

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Values of F at the probability level of: 5 %	1 %
Fats	4	410.452	102.6131	12.33	2.87	4.43
Human subjects ..	5	215.495	43.0990	5.18	2.71	4.10
Fat × subject ..	20	166.658	8.3229	6.38	1.93	2.55
Error	30	39.155	1.3052			
Total ..	59	831.760				

Once again the conclusions drawn are similar, viz., (1) fats are unlike to a statistically significant degree in respect of fat excretion, (2) human subjects react differently to different fats.

By carrying out another analysis after the exclusion of figures relating to vanaspati, m.p., 41°C., it is found that the remaining fats do not differ from each other. Interaction between fats and subjects is significant at 5 per cent. but not at 1 per cent. probability level after the exclusion of data relating to vanaspati, m. p., 41°C.

The conclusion to be drawn from both these analyses is that vanaspati, m. p., 41°C., is associated with a statistically significant higher degree of fat excretion and that the different subjects react in different manner towards these fats.

In respect of nitrogen, phosphorus and calcium metabolism, the figures of absorption (intake — excretion) have been analysed.

The mean figures of absorption are shown in Tables 81, 82 and 83.

Tale 81: Absorption of calcium (in g.) for four-day period

Subject	Vanaspati, m. p., 37°C	Ghee	Vanaspati, m. p., 41°C	Raw groundnut oil	Refined groundnut oil	Total
R M K	—0.3982	—0.1099	—0.7365	0.0548	0.0618	—1.1280
C S	—0.7794	—0.0390	—0.4109	—0.0193	—0.0304	—1.2790
N K	0.0260	0.2106	0.0053	0.2769	—0.0403	0.4785
B K	1.0108	1.0183	0.4654	0.9396	0.4491	0.8832
T N	0.2578	0.4377	0.0005	0.2856	0.2854	1.2670
M T	0.4530	0.0655	—0.3794	0.1686	—0.3715	—0.0638
Total ..	0.5700	1.5832	—1.0556	1 7062	0.3541	3.1579
Mean ..	0.0475	0.1319	—0.0880	0.1422	0.0295	

Table 82: Absorption of nitrogen (in g.) for four-day period

Subject	Vanaspati, m. p., 37°C	Ghee	Vanaspati, m. p., 41°C	Raw groundnut oil	Refined groundnut oil	Total
R M K	6.4420	3.3077	0.2130	6.7460	2.0689	19.0776
C S	5.9603	7.2383	10.4146	10.1266	9.6850	43.4248
N K	10.4205	10.1379	9.3975	10.3106	8.1031	48.3696
B K	14.4727	17.9021	13.2557	14.0825	12.2171	71.9301
T N	11.4778	14.6819	12.6379	11.7239	11.6004	62.1219
M T	14.3319	13.0025	12.5029	12.1335	11.2029	63.1737
Total ..	63.4052	66.2704	58.426	65.1231	54.8774	308.0877
Mean ..	5.2838	5.5225	4.8685	5.4269	4.5731	

Table 83: Absorption of phosphorus (in g.) for four-day period

Subject	Vanaspati, m. p., 37°C	Ghee	Vanaspati, m. p., 41°C	Raw groundnut oil	Refined groundnut oil	Total
R M K	0.2567	0.1959	—0.7156	0.1936	—0.1173	—0.1967
C S	—0.2911	—0.3879	—0.5135	—0.185	—0.0205	—1.3986
N K	0.3086	0.9295	0.4461	0.5217	0.3134	2.5193
B K	1.3793	1.5480	0.7926	1.1419	0.7900	5.6518
T N	0.8420	1.4109	0.6757	0.8775	0.7725	4.4786
M T	0.9695	0.7569	0.6103	0.6027	0.7371	3.6775
Total ..	3.4650	4.4542	1.2956	3.0509	2.4752	14.7409
Mean ..	0.2888	0.3712	0.1080	0.2542	0.2063	1.2284

In the case of calcium a negative balance has been recorded only in respect of vanaspati, m. p., 41°C. The results of statistical analysis are summarised in Tables 84—86.

Table 84: Analysis of variance of calcium absorption

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Expected value of F at probability level of 5%
Fats	4	0.415643	0.103911	3.69	2.87
Human subjects ..	5	1.816390	0.363278	12.90	2.71
Fat × subject	20	0.563328	0.028166		
Error	30	0.610279	0.020340		
Total ..	59				

Table 85: Analysis of variance of nitrogen absorption

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at the probability level of 5%
Fats	4	7.7323	1.9331	1.13	2.87
Human subjects ..	5	179.2573	35.8515	21.01	2.71
Fat × subject	20	34.1345	1.7067		
Error	30	19.0088	0.6336		
Total ..	59				

Table 86: Analysis of variance of phosphorus absorption

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at the probability level of 5%
Fats	4	0.458373	0.114593	4.38	2.87
Human subjects ..	5	3.763948	0.752789	28.78	2.71
Fat × subject	20	0.523062	0.026153		
Error	30	0.522563	0.017419		
Total ..	59				

The analysis of the data establishes that there is a difference between the fats in respect of calcium and phosphrus absorptions.

Calcium metabolism—An analysis was done excluding the figures for vanaspati, m. p., 41°C., which had given a negative absorption, and the result is given in Table 87.

Table 87: Analysis of variance of calcium absorption for four fats, viz., vanaspati, m. p., 37°C., ghee, groundnut oil and refined groundnut oil

Source of variation	Degrees of freedom	Sum of squares	Variance	F (observed)	Value of F at the probability level of 5 %
Fats	3	0.119126	0.039709	1.21	3.29
Human subjects ..	5	1.444620	0.288892	8.81	2.90
Fat × subject ..	15	0.492034	0.032802		
Error	24	0.490772	0.020448		
Total ..	47				

The recorded value of calcium absorption in the case of vanaspati, m. p., 41°C., is therefore, significantly lower than that of any one in the remaining four fats. Apart from this there is no evidence of a significant difference amongst the remaining four fats.

Nitrogen metabolism—The mean values of nitrogen absorption are given in Table 88.

Table 88: Mean values of absorption of nitrogen (in g.)

Fat	Mean	Standard error of mean
Vanaspati, m. p., 37°C.	5.284	+0.377
Ghee	5.523	+0.377
Vanaspati, m. p., 41°C.	4.861	+0.377
Raw groundnut oil	5.427	+0.377
Refined groundnut oil	4.573	+0.377

Refined groundnut oil has recorded the lowest value and ghee the highest value of nitrogen absorption. The differences between these values are not statistically significant.

Phosphorus metabolism—The mean values of phosphorus absorption are shown in Table 89.

Table 89: Mean values of absorption of phosphorus (in g.)

Fat	Mean	Standard error of mean
Vanaspati, m. p., 37°C.	0.28875	+0.466
Ghee	0.37118	+0.466
Vanaspati, m. p., 41°C.	0.10797	+0.466
Raw groundnut oil	0.25424	+0.466
Refined groundnut oil	0.20627	+0.466

Table 89 indicates that absorption of phosphorus was significantly high in the case of ghee and significantly low in the case of vanaspati, m. p., 41°C. Amongst the remaining three fats there is no evidence of any difference.

SECTION III

Feeding Experiments on children in Institutions

Children feeding experiments were conducted at three centres viz., (1) Aryan Orphanage, Delhi ; (2) David Sassoon Industrial School, Bombay ; and (3) St. Philomena's Orphanage and Good Shepherd Convent, Mysore. The results of statistical analysis are set out below separately for these three centres.

ARYAN ORPHANAGE, DELHI.

Boys and girls were treated separately. Each sex group was divided into two sub-groups, one kept on raw groundnut oil and the other on vanaspati, m.p., 37°C. In respect of each child, height and weight measurements were recorded at more or less regular intervals. The number of human subjects was not constant throughout the experiment as some individuals left the Institution and others were added to the group subsequently. For purposes of statistical analysis, only those children have been considered who were continuously under observation from 15th December 1947 to 13th December 1948, i.e., for a period of at least one year. As children differed from each other in regard to age, initial weight or height, it was considered necessary, as a preliminary step, to examine whether any of these factors could have influenced the relative increase in weight or height during the experimental period. Partial regression co-efficients of increase in height or weight on either age or initial weight or initial height were found to be statistically insignificant. It was recognised that since the numbers of subjects of the two sexes were neither equal nor proportional in the two treatment groups the data were rendered non-orthogonal. However, as the numbers involved were quite large the approximate method of analysis of weighted mean squares was adopted. A straightforward analysis of variance on the annual increase in height and weight was, therefore, carried out. Table 90 sets out the average increases in height and weight of the four groups, followed by Tables 91 and 92 showing the results of the analysis of variance.

Table 90 : Average increase in weight and height of boys and girls in Aryan Orphanage

Sex	Fat	Weight		Height	
		Number of Subjects in the group	Average increase in weight (in lbs.)	Number of subjects in the group	Average increase in height (in inches)
Boys	Raw groundnut oil	22	6.82	20	1.48
	Vanaspati, m.p., 37°C.	18	4.83	13	1.45
Girls	Raw groundnut oil	8	10.38	9	1.28
	Vanaspati, m.p., 37°C.	16	6.06	16	1.39

Table 91 : Analysis of variance of increase in weight

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of :	
					5%	1%
Sex	1	68.2380	68.2380	4.650	4.00	7.08
Fat	1	119.4110	119.4110	8.136	4.00	7.08
Sex × fat	1	18.7788	18.7788	1.280	4.00	7.08
Within	60	880.5852	14.6764			

Table 92 : Analysis of variance of increase in height

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	Value of F at the probability level of :	
					5%	1%
Sex	1	0.21607	0.21607	1.3948	4.02	7.12
Fat	1	0.01593	0.01593	0.0291	4.02	7.12
Sex × fat	1	0.05693	0.05693	0.1040	4.02	7.12
Within	54*	29.55287	0.54728			

*The number of children for whom height records are available is less than the number for whom weights have been recorded.

From the foregoing analyses the conclusion drawn is that a statistically significant difference is established between raw groundnut oil and vanaspati in so far as increase in weight among the inmates of the orphanage in Delhi is concerned.

It must be pointed out that the increase in weight was less in the case of vanaspati fed group than that of the group receiving raw groundnut oil.

Measurements on height fail to bring out this difference.

DAVID SASSOON INDUSTRIAL SCHOOL, BOMBAY

Feeding experiment on boys was carried out for one year, i.e., from the 20th October, 1947 to 20th October, 1948 on the inmates of the David Sassoon Industrial School, Bombay. The ages of the boys varied from 11 to 17 years.

According to a nutrition survey carried out in the beginning of the experiment, the boys were divided into two groups namely normal and below-normal. From each of these two groups, 2 batches were formed similar to each other by a process of random selection. One batch from the normal groups and one batch from the below-normal group were selected at random and fed on vanaspati, the remaining 2 batches were marked for oil feeding. Although care was taken to ensure similarity of groups at the beginning, a number of boys are reported to have left the School during the course of the experiment. For purposes of statistical analysis, only those boys in each group have been considered for whom continuous records are available for the entire experimental period. Height and weight measurements of these boys were recorded. It is stated that except for the difference in fats there was no material variation in the proximate principles in the diets consumed by the various groups.

The mean gains in weight and height recorded in the case of each of the 4 groups are shown in Table 93.

Table 93 : Average increase in weight and height in the four groups

Group	Number of boys	Mean increase in weight (in lbs.)	Mean increase in height (in inches)
Normal boys getting oil	38	7.355	1.592
Normal boys getting vanaspati	38	8.947	1.668
Below-normal boys getting oil..	43	8.430	1.772
Below-normal boys getting vanaspati	43	9.256	1.562

Before the statistical significance of these differences can be assessed it is necessary to examine how far increases in height or weight were conditioned by the initial measurements of each boy and his age. The analysis of covariance given in Table 94 summarises the results of this study.

Table 94 : Analysis of co-variance between increase in weight and age or initial weight

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	5% F
Due to fitted regression ..	2	119.66	59.83	3.30	3.04
Error	156	2831.19	18.15		
Total ..	158	2950.85	18.68		

It is clear that age and initial weight have significantly affected the increase in weight. Therefore, the growth in weight for each subject has been adjusted for the variation in age and initial weight.

Table 95 gives the adjusted average values for increase in weight.

Table 95 : Adjusted average values for increase in weight

	Normal	Below-normal
Oil	7.278	8.679
Vanaspati	8.924	9.099

An analysis of variance between the four groups, viz., (*i*) vanaspati—normal; (*ii*) vanaspati—below-normal; (*iii*) oil—normal and (*iv*) oil—below-normal, has been carried out on adjusted figures and the results are summarised in Table 96.

Table 96 : Analysis of variance of increase in weight

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	5% F
1. Fats	1	45.80	45.80	2.52	3.90
2. Normal *vs.* Below-normal	1	332.97	332.87	18.34	3.90
3. Interaction between (1) and (2)	1	1.64	1.64		3.90
4. Error	156	2831.19	18.15		

The exact method no doubt would have been the analysis of residual variance adjusting for the regression in the treatment and treatment+error lines. The above method was adopted as being simpler to follow and the conclusions are not suspected to be affected by the approximation involved. This procedure has been adopted in all similar cases in the course of this study.

From Table 96 it is evident that the difference in the increase in weight as observed between the two fat groups is not significant.

In order to investigate whether age and initial height affected the increase in height an analysis of co-variance of increase in height with age and initial height was carried out. The results are shown in Table 97.

Table 97 : Analysis of co-variance between increase in height and age or initial height

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	5% F
Regression on age and initial height.	2	11.80	5.9000	11.85	3.04
Error	156	77.65	0.4978		
Total	158	89.55	0.5661		

It is evident that the age together with initial height affected significantly the variation in height. Therefore, adjustments have been made for this variation and the adjusted value for increase in height is given in Table 98.

Table 98 : Adjusted average values for increase in height

	Normal	Below-normal
Vanaspati	1.631	1.562
Raw groundnut oil	1.636	1.715

The significance of the difference between vanaspati and oil groups in the case of normal as well as the below-normal boys has been assessed. Table 99 shows the analysis of the variance between these groups.

Table 99 : Analysis of variance of growth in height

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	5% F
1. Fats	1	0.1333	0.1333	0.268	3.90
2. Normal *vs.* Below-normal	1	0.0798	0.0798	0.160	3.90
3. Interaction between (1) and (2)	1	0.6195	0.6195	1.244	3.90
4. Error	156	77.6500	0.4978		

The boys in the normal as well as the below-nornal groups do not show any significant differences in increase in height when treated with vanaspati or raw groundnut oil.

ST. PHILOMENA'S ORPHANAGE AND GOOD SEPHERED CONVENT, MYSORE

The children selected for the feeding trials of vanaspati and raw groundnut oil were inmates of two orphanages in Mysore. They were all below 15 years of age and their weights ranged from 25 to 80 lbs. They had been on poor rice diet getting less than 1 oz. of fat per day before the commencement of the experiment. The experiment began in July 1947 and terminated in July 1948, the period of experimentation being one year. The total numbers of children available with complete records of weight and height were 109 and 145 respectively. Each of these two sex groups was divided into two groups on a random basis, one group being allotted to vanaspati and the other to raw groundnut oil.

In respect of each child the age, initial weight, final weight and clinical scores for eyes and skin in July 1947 and July 1948 were recorded as also the initial and final heights.

For the purpose of assessing the difference in the effects of the two fats, the subjects belonging to either sex were divided into two groups on the basis of age. The two groups were (1) children of age 9 years and under and (2) children of age above 9 years.

The average increases in weight in the different age groups of boys and girls for the two fats are shown in Table 100.

Table 100 : Average increase in weight of boys and girls in different age groups

Age group	Raw Groundnut oil		Vanaspati, m.p., 37°C.	
	Number of subjects	Mean increase in weight (lbs.)	Number of subjects	Mean increase in weight (lbs.)
Boys				
9 years and under	6	2.938	6	4.250
Above 9 years	23	4.728	22	7.705
Girls				
9 years and under	7	4.643	10	3.888
Above 9 years	16	6.125	19	8.007

Statistical analysis has been carried out separately for each sex group. The results are summrised in the two analyses of variance given in Tables 101 and 102.

Table 101 : Analysis of variance of increase in weight for boys

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	5°/₀F
Fats	1	98.22	98.22	8.307	4.03
Age	1	64.96	64.96	5.495	4.03
Age × fat	1	6.56	6.56		
Error	53	626.69	11.82		

Table 102 : Analysis of variance of inrease in weight for girls

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	5 °/₀F
Fats	1	13.68	13.68	1.289	4.05
Age	1	102.44	102.44	9.655	4.05
Age × fat	1	19.42	19.42	1.831	4.05
Error	47	509.26	10.61		

It is clear from Tables 101 and 102 that in the case of boys the fats show a significant difference between themselves,vanaspati group showing a greater increase than the oil group.

In the case of girls the difference between the two fats is not significant. A further examination showed that both boys and girls below the age of 9 years did not exhibit any significant difference for the two fats. It is at the higher age groups (above 9 years) that a significant difference between the two fats is established for both the sexes in favour of vanaspati. These results emerge from the two analyses of variance given in Table 103.

Table 103 : Analysis of variance of increase in weight of boys and girls

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	5°/₀F
9 years and under					
Fats	1	0.0961	0.0961	0.0198	4.24
Sex	1	2.4674	2.4673	0.482	4.24
Fats × sex	1	7.4213	7.4213	1.450	4.24
Error	25	127.9571	5.1182		
Above 9 years					
Fats	1	124.4877	124.4877	9.386	3.99
Sex	1	13.4693	13.4693	1.016	3.99
Fats × sex	1	5.8690	5.8690	0.433	3.99
Error	76	1007.9897	13.2630		

Vanaspati, therefore, shows a significantly higher increase in weight for children above the age of 9 years. There is no evidence of such a difference for the children of lower ages.

A parallel statistical analysis was carried out on the data of increase in height. For this purpose, each sex group was divided into two age-groups (*i*) 9 years and under (*ii*) above 9 years. Table 104 gives the average increase in height of boys and girls in different age groups separately for the two fats.

Table 104 : Average increases in height of boys and girls in different age groups

Age group	Raw groundnut oil		Vanaspati, m.p., 37°C.	
	Number of subjects	Mean increase in height (in inches)	Number of subjects	Mean increase in height (in inches)
Boys				
9 years and under	8	2.312	9	2.001
Above 9 years	31	2.831	29	2.730
Girls				
9 years and under	14	2.009	10	2.315
Above 9 years	19	1.751	25	2.061

The figures have been analysed separately for the two sexes and the results are shown in Table 105.

Table 105 : Analysis of variance of increase in height

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	5% F
Boys					
Fats	1	0.4170	0.4170	<1	3.99
Age	1	5.2120	5.2120	3.926	3.99
Age × fat	1	0.4164	0.1464	<1	3.99
Error	73	96.9007	1.3274		
Girls					
Fats	1	1.5842	1.5842	1.033	4.00
Age	1	1.0011	1.0011	0.653	4.00
Age × fat	1	0.0002	0.0002		4.00
Error	64	98.1556	1.5337		

The two fat groups do not show any significant difference in either case.

Further examination of the differences between the two fats was made separately for each age or sex group. In no case was a significant difference found between the two fats in respect of increase in height. The results of analysis are sumarised in the Table 106.

Table 106 : Analysis of variance of increase in height for children

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F (observed)	5% F
9 years and under					
Fat	1	0.0214	0.0214	<1	4.11
Sex	1	0.0003	0.0003	<1	4.11
Fat × sex	1	0.9345	0.9345	0.662	4.11
Error	37	52.1806	1.4103		
Above 9 years					
Fats	1	0.1308	0.1308	..	3.96
Sex	1	18.7027	18.7027	13.090	3.96
Fat × sex	1	1.0612	1.0612	..	3.96
Error	100	142.8758	1.4288		

The conclusion drawn is that their is no evidence of a difference in the increases in height in the case of vanaspati and raw groundnut oil.

Another analysis was carried out by classifying the children according to their initial weights. They were divided into three weight groups as follows :

Group I : Children with initial weights below 50 lbs.

Group II : Children with initial weights between 50-70 lbs.

Group III : Children with initial weights above 70 lbs.

Each of these groups was further sub-divided into those given raw groundnut oil and those given vanaspati, m.p., 37°C. Table 107 gives the mean increase in weight and height of the groups.

Table 107 : Average increase in weight and height of boys and girls

	Weight		Height	
	Number of subjects	Mean increase (in lbs.)	Number of subjects	Mean increase (in inches)
Boys				
Vanaspati, m.p., 37°C.				
Group I	10	4.788	16	2.681
Group II	13	6.971	16	2.903
Group III	5	11.300	6	2.908
Raw groundnut oil				
Group I	11	2.852	16	2.469
Group II	14	5.446	16	3.070
Group III	4	4.688	7	2.519
Girls				
Vanaspati, m.p., 37°C.				
Group I	15	4.408	18	2.071
Group II	18	8.937	10	2.576
Group III	6	8.896	7	1.661
Raw groundnut oil				
Group I	11	4.931	20	1.963
Group II	6	8.042	7	2.647
Group III	6	4.667	6	0.900

As the number of children varied considerably from group to group a series of 't' tests were carried out to ascertain the significance of the differences existing between the average increses in height or weight in vanaspati *versus* groundnut oil groups. The results are summarised in Table 108.

Table 108: Results of 't' test of significance between group means of vanaspati and groundnut oil.

Comparison between mean of	Weight measurements			Height measurements		
	't'	Degrees of freedom	Remarks	't'	Degrees of freedom	Remarks
			Boys			
Group I	2.256	19	Significant at 5% level, vanaspati greater than oil	1.173	30	Not significant
Group II	0.957	25	Not significant	0.341	30	Not significant
Group III	6.128	7	Significant at 1% level, vanaspati greater than oil	0.784	11	Not significant
			Girls			
Group I	0.573	24	Not significant	0.027	36	Not significant
Group II	0.446	12	Not significant	0.133	15	Not significant
Group III	1.921	10	Not significant	2.208	11	Significant at 5% level, vanaspati greater than oil

In regard to mean increase in weight the differences between vanaspati nd raw groundnut oil groups are found to be significant among boys of roups I and III. In each case the results are in favour of vanaspati. n the case of height measurements the difference emerges into statistical gnificance only in the case of Group III (girls) and vanaspati is associated ith a higher rate of increase.

Statistical analysis of clinical symptoms—For the purpose of statistical nalysis both boys and girls were divided into two groups viz., Group I consting of children who had no clinical symptoms (whose score was zero) hen the experiment started, and Group II consisting of the remaining ildren whose score was higher than zero.

A separate analysis was done for each group to assess the significance the difference between the effects of the two fats. The numbers of child- n whose clinical scores changed at the end of the experiment are shown Tables 109 and 110.

Table 109 : Number of children with zero clinical score at the beginning of the experiment (Group I)

	Number of children at the commencement of the experiment		Number of children in whom clinical symptoms appeared	
	Eyes	Skin	Eyes	Skin
naspati	15	22	4	12
w groundnut oil	14	25	4	8

Table 110: Number of children with a clinical score higher than zero at the beginning of the experiment (Group II)

	Number of children at the commencement of the experiment		Number of children in whom symptoms disappeared	
	Eyes	Skin	Eyes	Skin
Vanaspati	42	35	18	16
Raw groundnut oil	38	27	11	9

Table 111 gives the results of the statistical tests carried out on the combined figures of boys and girls in respect of the differences in clinical symptoms under the two fats.

Table 111 : X^2-test for symptoms of eyes and skin

Group	Value of X^2	5% X^2
Symptoms of eyes		
I. With zero clinical score in July 1947	0.013	3.841
II. With clinical score higher than zero in July 1947 ..	0.670	3.841
Symptoms of skin		
I. With zero clinical score in July 1947	2433	3.841
II. With clinical score higher than zero in July 1947 ..	0.949	3.841

There is no evidence of any difference between the two fats in so far as the manifestations of clinical symptoms are concerned.

SUMMARY

ANIMAL EXPERIMENTS

Experiments were carried out on rats at four centres viz., (*i*) Indian Dairy Research Institute, Bangalore ; (*ii*) Indian Institute of Science, Bangalore ; (*iii*) Indian Veterinary Research Institute, Izatnagar and (*iv*) University College of Science and Technology, Calcutta with five fats viz., raw groundnut oil, refined groundnut oil, vanaspati, m.p., 37°C. vanaspati, m.p., 41°C. and ghee on the growth, reproductive and lactating capacities and vitamin A metabolism of the second and third generations of rats. At each centre, the effect of each fat was tested on groups of 12 rats consisting of six males and six females. Different basal diets viz., synthetic diet, poor rice diet with or without different suplements and poor Bengali diet were used for the experiments.

Growth experiments—The statistical analysis reveals that in all the four centres ghee is associated with the highest gain in weight. Among the remaining four fats vanaspati, m.p., 41°C. shows the highest rate of growth at three centres viz., University College of Science and Technology, Calcutta, Indian Dairy Research Institute, Bangalore and Indian Veterinary Research Institute, Izatnagar. On the other hand, experiments carried out at the Indian Institute of Science, Bangalore show the lowest rate of growth for the same fat.

Data relating to the second and third generations of rats were statistically analysed for two centres viz., Indian Institute of Science, Bangalore and Indian Veterinary Research Institute, Izatnagar. The experiments conducted at Izatnagar show the highest rate of increase in weight for the ghee group in the second and third generations. Vanaspati, m.p., 41°C. shows the lowest rate of increase for rats of the second generation. The Bangalore experiments show that for rats of the second and third generations, vanaspati, m.p., 37°C. produced the highest increase in weight.

Experiments on fertility—Experiments were carried out at all the four centres to test the relative values of ghee, vanaspati and groundnut oils on fertility. This has been measured in two ways :

(*i*) the percentage of female rats becoming pregnant under comparable conditions, and

(*ii*) the average size of litter born per female rat.

It has been found that ghee is associated with the highest fertility. The remaining four fats viz., vanaspati, m.p., 37°C. or m.p., 41°C. and raw or refined groundnut oils do not show any statistically significant differences in fertility.

Lactating capacity—A ratio was worked out of the number of new born rats surviving 20 days after birth to the total number of fertile mother rats in each diet and fat group. Figures have been studied for three centres viz., (*i*) Indian Dairy Research Institute, Bangalore. (*ii*) Indian Institute of Science, Bangalore and (*iii*) Indian Veterinary Research Institute, Izatnagar. In no case has any difference been established in the five fats in respect of lactating capacity.

Fat content of liver—The experiments carried out at the Indian Institute of Science, Bangalore show that vanaspati, m.p., 41°C. is associated with the highest content of fat in liver, the figure being significantly higher than those for ghee, raw or refined groundnut oil. Vanaspati, m.p., 37°C. differs in regard to the fat content of liver from ghee or the two oils in the third generation only, being associated with the largest amount of fat in liver. At the University College of Science and Technology, Calcutta, where fat content of liver was studied on only one generation, the results are in conformity with those of Bangalore, vanaspati, m.p., 41°C. producing the highest fat content of liver.

Vitamin A content of liver—Vitamin A content of liver was studied for three generations of rats in the Indian Institute of Science, Bangalore and for one gneration in the University College of Science and Technology, Calcutta and the Indian Dairy Research Institute, Bangalore. In no generation has any significant difference been established at any of the three places among different fats.

METABOLISM STUDIES ON ADULT HUMAN SUBJECTS AND RATS

Metabolism studies were carried out at the Nutrition Research Laboratories, Coonoor on adult male workers and at the Indian Institute of Science, Bangalore, on adult males as well as on children below the age of 11 years and on adult rats to ascertain whether the fats were significantly different form one another in respect of the amount of absorption of fat, phosphorus, nitrogen and calcium.

At Coonoor it is observed that out of the five fats tested viz., vanaspati, m.p., 41°C. vanaspati, m.p., 37°C. raw groundnut oil, refined groundnut oil and ghee, vanaspati, m.p., 41°C. caused the lowest degree fat absorption and significantly different from the other four fats. No difference is established among the four fats viz., vanaspati, m.p., 37°C. raw groundnut oil, refined groundnut oil and ghee. In regard to the relative effects of different fats on calcium, phosphorus and protein metabolism in human subjects, calcium showed a negative balance in respect of vanaspati, m.p., 41°C. In regard to differences among the remaining fats in respect of calcium metabolism, no definite conclusions are possible on this limited experience. Statistically significant differences are found to exist among the five fats in respect of phosphorus and nitrogen metabolism. The highest phosphorus absorption has been recorded in the case of ghee and lowest in the case of vanaspati, m.p., 41°C. These differences are statistically significant. Amongst the remaining three fats viz., vanaspati, m.p., 37°C., raw groundnut oil and refined groundnut oil, no definite conclusion can be drawn in respect of phosphorus absorption.

The nitrogen absorption values for vanaspati, m.p., 41°C. and also for refined groundnut oil are found to be significantly lower than those for ghee. However, no difference has beeen established between the nitrogen absorption value for vanaspati,m.p., 37°C. on one hand and that for ghee or raw groundnut oil on the other.

In the metabolism experiments conducted at the Indian Institute of Science, Bangalore, with five fats viz., raw groundnut oil, refined groundnut oil, vanaspati, m.p., 37°C., vanaspati, m.p., 41°C. and ghee on human adults, it is observed that the largest amont of faecal excretion occurred in the group receiving vanaspati, m.p., 41°C. the next largest being observed

in the group fed on vanaspati, m.p., 37°C. Further vanaspati,m.p., 41°C. has shown the lowest degree of fat absorption the next in order being vanaspati, m.p., 37°C. At this centre no statistically significant difference has been established among the fats in respect of calcium metabolism. Differences in regard to phosphorus and nitrogen metabolism also are significant only at 5 per cent probability level but not at the 1 per cent probability level of significance.

The analysis of the iodine numbers of plasma fatty acids of subjects fed on different fats shows a significant degree of variation in so far as the values are significantly high for both raw and refined groundnut oils and low for both vanaspati, of m.p., 41°C. and m.p., 37°C. as well as for ghee. Further, there is no difference of significance between the iodine numbers for ghee group on one hand and those for vanaspati of either of the two melting points on the other.

At the Indian Institute of Science, Bangalore, metabolism studies were also carried out on children below the age of 11 years. They were given diets supplemented with vanaspati, m.p., 37°C. or raw groundnut oil. The two fat groups failed to bring out any significant difference in regard to calcium and nitrogen metabolism but there is an indication that phosphorus absorption is greater in the vanaspati group than in the oil group.

Metabolism studies on young rats were also conducted at the Indian Institute of Science, Bangalore. The diets of the rats were supplemented either with vanaspati, m.p., 7°C. or butter. Data on calcium and phosphorus absorption and faecal excretions were studied at three stages viz., 3 weeks, 6 weeks and 9 weeks of rat's age. No difference between vanaspati and butter groups has been established.

The question as to whether 15 per cent casein diet supplemented with vanaspati, m.p., 37°C. affected the absorption of calcium or phosphorus in comparison with the supplementation by other fats such as ghee, raw groundnut oil or coconut oil was also studied at the Indian Institute of Science, Bangalore by experimenting with rats. The differences among fats are found to be significant, phosphorus absorption being the highest in the case of vanaspati, m.p., 37°C. and lowest for ghee. The mean absorption of vanaspati, m.p., 37°C. and groundnut oil is of the same order of magnitude. Calcium absorption is significantly lower in the case of ghee than for the other fats, no sigificant difference being observed among the remaining three fats.

INSTITUTION FEEDING EXPERIMENTS

These experiments were carried out in order to find whether vanaspati, m.p., 37°C. had any deleterious effect in comparison to raw groundnut oil. Along with other observations in this connection, the changes in height and weight of the children were recorded.

The experiments conducted at the Aryan Orphanage, Delhi show that increase in weight was significantly less in the group of children fed on vanaspati, m.p., 37°C. as compared with children receiving raw groundnut oil. Measurements of height fail to bring out any statistically significant difference between the two fats.

The feeding experiments conducted at the two Mysore orphanages show, on the other hand, a significant difference between the two fats in the case of children above nine years of age ; those receiving vanaspati, m.p., 37°C. recording a significantly higher rate of growth than those receiving raw groundnut oil. The weight records of children of 9 years and under fail to bring out any significant difference between the two fats.

Analysis carried out separately on each sex group shows that in the case of boys the fats show a significant difference between themselves, vanaspati being associated with a greater increase in weight than raw groundnut oil. In the case of girls, however, the difference between the two fats is not statistically significant.

The analysis fails to bring out any difference in the increase in height in the case of vanaspati and oil groups.

No difference between the two fats is observed in respect of manifestations of clinical symptoms.

The experiments conducted at the David Sassoon Industrial School, Bombay fail to bring out any significant difference in increase in weight or height in the vanaspati and oil groups.

From this summary of conclusions it is apparent that there are inconsistencies and even contradictory results in some of the experiments. However, the statistical analysis of the results leads to the following broad conclusions :

(A) Vanaspati, m.p., 41°C. is associated with a negative balance of calcium absorption. Further it is found that vanaspati, m.p., 41°C. leads to : (*i*) a reduced absorption of other nutritive principles including the fat itself ; (*ii*) the highest fat content of liver which is, however, within the normal range; and (*iii*) the highest degree of faecal excretion.

(B) The relative nutritive values of the fats studied are as follows in the descending order : (*i*) ghee; (*ii*) raw groundnut oil, vanaspati, m.p., 37°C and refined groundnut oil, which can be grouped together; and (*iii*) vanaspati, m.p., 41°C.

PART II

INTRODUCTION

Realising the importance of scientific research in dealing with the problems of the vegetable oil Industry in India, the Indian Vanaspati Manufacturers' Association generously placed a substantial sum of money unconditionally at the disposal of the Council of Scientific and Industrial Research for furtherance of work in the field. The Council thankfully accepted the offer and appointed a Vanaspati Research Advisory Committee consisting of the following members to scrutinise research programmes and to make the necessary recommendations to the Council :

Sir S. S. Bhatnagar (Chairman), Director, Scientific and Industrial Research and Secretary, Ministry of Natural Resources and Scientific Research, New Delhi; Dr. B. C. Guha, Member, Damodar Valley Corporation, Calcutta; Dr. J. N. Mukherjee, Director, Central Building Research Institute, Roorkee; Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories Coonoor ; Dr. V. Subrahmanyan, Director, Central Food Technological Research Institute, Mysore; and Mr. S. H. Turner, Factory Manager, Hindustan Vanaspati Manufacturing Co. Ltd., Bombay.

In their first meeting, the Vanaspati Research Advisory Committee discussed the different aspects of researches on vanaspati and the type of problems facing the vanaspati manufacturers. The important points of discussions are outlined below:

(*a*) Vanaspati can be prepared by one of the following methods, viz., (*i*) *straight hardening* : according to which the whole batch of the oil can be hardened to a desired consistency ; (*ii*) *blending* : according to this procedure a portion of the oil is hydrogenated to a high degree so that the melting point may be between 45°C. and 60°C. This hardened product is then melted together with varying volumes of refined oil and the mixture then allowed to cool under proper conditions.

Of the two processes, the one involving blending is stated to be cheaper and more flexible. It may facilitate greater output of vanaspati, because one part of hardened oil of m. p., 60°C. could be blended of wtih about seven parts of refined oil to obtain a blend of m. p., 37°C. The blended product also contains a higher percentage of essential unsaturated fatty acid glycerides naturally occurring in the oil.

So far, most of the studies on the nutritive value of vanaspati have invariably been carried out on the straight hardened product. It is of considerable practical interest to compare the straight hydrogenated product with the blended one as regards its nutritive value. Hence, the need for exhaustive study from the physiological point of view, taking into account the chemical and physical conditions of straight hardened vanaspati, was brought to the notice of the Committee.

(*b*) It was pointed out that no work had been done on chemical

analyses of various hydrogenated fats and their corresponding crudes and therefore a survey of the various hydrogenated products for their *iso*-oleic and linoleic acids contents, nickel content and proportion of minor constituents, viz., sterols, tocopherols, phosphatides and lecithins in the crude oils and the manufactured products would be of great value. As this study is both important and comprehensive, it was considered desirable that co-ordinated work should be done in more than one labortory.

(*c*) The need for animal experiments for studying the problem of toxicity of nickel and nickel soaps was also discussed with special reference to the state of division, physical condition and nickel balance and also whether the effect was cumulative. It was also considered that the question of *iso*-oleic acid from the nutrition point of view was important and that the various fractions should be isolated and studied.

(*d*) The need for studying the stability of crude and refined oils and hydrogenated fats of different melting points, nickel contents and acidity at different temperatures was discussed. It was also considered that the stability of added carotene and vitamin A, with and without antioxidants and also with and without added sesame oil, should be studied taking into consideration freedom from rancidity, peroxide value, Kreis test etc.

The Committee then discussed the allocation of work between various laboratories and the following schemes of researches on vanaspati were sponsored.

(1) **"Investigations from the physiological points of view on straight hardened and blended vanaspati"** to be carried out at the Indian Institute of Science, Bangalore, under Dr. V. Subrahmanyan.

(2) **" Determination of *iso*-oleic and linoleic acids, nickel contents, and minor constituents in various hydrogenated products and in their crudes"** to be carried out at two laboratories—(*i*) Indian Institute of Science, Bangalore, under Mr. B. N. Banerjee and (*ii*) Department of Chemical Technology, University of Bombay, under Dr. J. G. Kane.

(3) **"Investigations on the toxicity of nickel and nickel soaps and the question of *iso*-oleic acid from the nutrition point of view"** to be carried out at the Nutrition Research Laboratories, Coonoor, under Dr. V. N. Patwardhan.

(4) **"Investigations on the stability of crude and refined oils and hydrogenated fats of different melting points, nickel contents and acidity and problems on vitamin A and carotene-fortification"** to be carried out at the University College of Science and Technology, Calcutta, under Dr. B. C. Guha.

Progress reports from the different laboratories were submitted to the Council of Scientific & Industrial Research. Meetings of the Vanaspati Research Advisory Committee were held from time to time and the progress of the work was discussed. The results obtained, which are of practical interest, are summarised below.

(*a*) *In vitro* studies by enzymic digestion, metabolism studies in rats as well as in children and chylomicrographic studies in children, have shown that blended samples of vanaspati of different melting points are digested at practically the same rate as straight hardened vanaspati of corresponding melting points. Institution feeding experiments also have revealed no difference between straight hardened vanaspati and blended sample of the same melting point.

(*b*) A comparison of various vanaspatis and their corresponding crudes in terms of linoleic acid, *iso*-oleic acids, nickel contents etc., has shown that linoleic acid content is appreciably reduced and *iso*-oleic acids are formed on hydrogenation. *Iso*-oleic acid content of various vanaspatis has been found to vary between 15 and 45 per cent. The nickel content of various factory samples of vanaspati has been found to lie between 0.1 and 0.5 parts per million, while that of market samples appears to be higher. In some cases, the nickel content has been found to be as high as 3 to 7 parts per million. Determination of tocopherols in raw vegetable oils and vanaspatis prepared from them has revealed that tocopherols are not destroyed to an appreciable extent during hydrogenation.

(*c*) Experiments conducted on rats as well as on monkeys to test the toxicity of nickel and nickel soaps present in vanaspati have shown that ingestion of nickel in far greater quantities than are found in vanaspatis does not have any harmful effect on animals. Nickel has been found to accumulate in certain tissues. The body is freed of accumulated nickel within about a month after the discontinuation of nickel feeding. Examination of the rats in which nickel has accumulated has not shown any abnormality. As regards the effect of *iso*-oleic acids, it has been observed that the growth of rats receiving *iso*-oleic acids is higher than that of rats receiving oleic acid. The digestibility of *iso*-oleic acids is only slightly lower than that of oleic acid. Rats are capable of utilising *iso*-oleic acids as efficiently as oleic acid for their metabolic purposes.

(*d*) Studies on the stability of raw, refined and hydrogenated groundnut oils have shown that the stability is greatest in the case of hydrogenated fats and the addition of ethyl gallate improves the keeping quality of oil or vanaspati samples. Refined sesame oil admixed with vanaspati to the extent of 5 per cent tends to lower the keeping quality of vanaspati. Samples of vanaspati fortified with carotene have been found to lose 33 per cent of carotene on 8 months' storage at 37°C. Addition of ethyl gallate has been found to reduce the loss of carotene considerably.

The results indicated that the linoleic acid content of hydrogenated oil varied from 0 to 10 per cent with an average of 5 per cent. The raw oil contained linoleic acid from 20 to 30 per cent. The reduction in the proportion of linoleic acid must be due to the changes effected by hydrogenation. The work on toxicity of nickel showed that the nickel in the tissue was in a most labile form and was easily thrown out and that there were no toxicity symptoms due to nickel. Investigations in the studies on stability of hydrogenated, refined and raw oils showed that raw sesame oil afforded better protection and colour to vanaspati than hydrogenated sesame oil and that vitamin A acetate was less stable than carotene when incorporated into vanaspati.

The research papers which follow relate to a substantial part of the work carried out under the auspices of the Committtee up to the beginning of 1951.

Studies on the nutritive value of blended vanaspati*

PART I—DIGESTIBILITY OF FATS

Vanaspati (shortening) with the desired m. p., 37°C., can be prepared by (1) straight hardening, according to which the whole batch is hardened to the required melting point, and (2) blending, according to which a portion of the oil (14-16 per cent in the case of groundnut oil) is hydrogenated to give a product with m.p., 45-60°C., and the product so obtained is blended with the refined oil. The blending process is said to be cheaper and more flexible.

Data relating to the quantities of straight hardened and blended vanaspati produced in India are not available. It is stated, however, that the majority of the vanaspati brands are straight hydrogenated products.

Extensive studies have been carried out on the nutritive value of straight hardened vanaspati [1—7] but information on the blended product is scanty. A comprehensive study of blended hydrogenated products has been undertaken in these laboratories, and the results on digestibility trials are reported in this paper.

Experimental.

Adult albino rats weighing about 150 g. were used as test animals. The experimental diet employed in these studies consisted of : casein, 15; fat, 15 ; sugar, 5 ; salt mixture, 4 ; and starch, 60 parts. The animals were fed *ad lib.*, each animal receiving in addition daily 1 cc. of adexolin diluted 10 times with groundnut oil, 0. 2g. of yeast (Squibb), 40 mg. of thiamine and 70 mg. of riboflavin.

The fats experimented with were : butter fat (ghee), coconut oil, refined groundnut oil, partially hydrogenated oils, and 3 blends of hydrogenated groundnut oil of m.p., 60° C. with refined groundnut oil, melting at 38° C., 45° C. and 51° C. respectively.

Experimental animals were allowed 5 days as 'period of orientation'. Faeces were collected after this period. Individual collections were made daily and excreta preserved in an ice chamber.

The combined faeces for the first 8-days period were dried at 60° C. to constant weight, ground to powder and extracted with ether. The residue was dried, ground to a paste with 50% sulphuric acid and the paste extracted with ether. The neutral fat and the fatty acids so obtained were weighed after drying at 60° C. The digestibility of the fats was calculated in the usual way and the results are given in Table 1.

* The work described in this section was carried out by Shri M.R. Sahasrabudhe and Dr. V. Subrahmanyan, Department of Biochemistry, Indian Institute of Science, Bangalore and Central Food Technological Institute, Mysore.

Table 1 : Digestibility of fats

	Ghee	Coconut oil	Groundnut oil, refined	Hydrogenated oil,* m.p., 38°C.	Blended product, m.p., 38°C.	Hydrogenated oil*, m.p., 45°C.	Blended product, m.p., 45°C.	Hydrogenated oil,* m.p., 51°C.	Blended product, m.p., 51°C.
Number of rats ..	8	8	8	8	8	6	6	6	6
Av. wt. of rats, g. ..	155	150	140	142	152	153	160	158	159
Av. change in body wt., g.	+3	+1	+4	+3	+4	+1	+0	−6	−4
Av. fat ingested, g. ..	9.5	10.0	9.8	10.1	9.2	10.2	10.8	9.0	9.1
Av. wt. of stools, g. ..	4.6	4.5	4.1	4.8	5.0	7.5	6-3	10.8	9.9
Av. total fat excreted corrected for metabolic fat, g. ..	0.36	0.42	0.47	0.38	0.32	0.846	1.10	3.54	3.12
Neutral fat and fatty acids, g.	0.22	0.29	0.30	0.22	0.25	0.42	0.34	0.38	0.38
Soaps	0.28	0.36	0.38	0.40	0.32	0.75	1.13	3.2	3.0
Coeff. of digestibility ** ..	96.2±1.2	95.3±1.3	95.7±1.08	96.2±1.2	96.5±2.6	92.3±1.5	90.2±0.8	60.5±1.4	66.3±1.6

* Straight hydrogenated groundnut oils were supplied by Messrs. Hindusthan Vanaspati Manufacturing Co. Ltd., Bombay

** Mean value

± Standard error of the mean

Table 2 : Percentage of saturated fatty acids in fats

	Ghee	Coconut oil	Groundnut oil, refined	Hydrogenated oil,* m.p., 38°C.	Blended product, m.p., 38°C.	Hydrogenated oil,* m.p., 45°C.	Blended product, m.p., 45°C.	Hydrogenated oil.* m.p., 51°C.	Blended product, m.p., 51°C.
Fat fed	57	84	18	30	34	38	40	60	65
Faecal fat **	58±1.0	58±1.2	20±0.96	38±1.8	48±1.1	54±0.88	56±1.2	72±1.0	75±2.0

*Straight hydrogenated groundnut oils were supplied by Messrs. Hindusthan Vanaspati Manufacturing Co. Ltd., Bombay.

** Mean value.

± Standard error of the mean

The fat present in the second 8-day experimental period was saponified with 15 per cent KOH, neutralized and extracted with ethyl ether. The fatty acids were partitioned into solid and liquid fatty acids according to Twitchell's lead-salt method. The results for saturated fatty acids present in the ingested fat are given in Table 2.

Discussion

The digestibility of oil is not affected by incorporating in it fully saturated fats in the blends melting within the physiological range.

The quantity of saturated fatty acids present in faecal fat is significantly higher than that present in the fat fed to the animal.

Analyses show no significant differences in the fats of faeces collected from animals fed on hydrogenated and blended fats with m.p. exceeding 45° C.

Saturated acids present in blends (m.p. 38°C.) tend to accumulate in faecal fat.

PART II—*In Vitro* HYDROLYSIS OF FAT BY PANCREATIC LIPASE

Hydrolysis of fats by lipolytic enzymes plays an important part in the absorption of fats from the intestine. The behaviour of various samples of vanaspati towards the pancreatic lipase was studied *in vitro*. It was aimed to find out the difference, if any, in the splitting by the pancreatic lipase of straight hardened vanaspati and a blended sample of the hydrogenated fat melting at the same temperature under a definite set of conditions. Rate of hydrolysis of cow ghee was taken as standard. Hydrolysis of a few samples of low grade ghee, melting at about 43°C., was also studied and compared with the blended vanaspati melting at the same temparature.

Experimental

Various blends were prepared by thoroughly mixing the two constituents in a liquid state and gradually cooling to room remperature and then in the Frigidaire. Rapid cooling in the Frigidaire was avoided since it was observed that when cooled gradually the blending is better. A blend of the particular melting point was prepared by the method of trial and error and hence it took a long time in getting the exact proportions of the two constituents in a particular blend.

In the case of high melting hydrogenated fats, emulsification was rendered difficult because of the tendency of the fat to salt out at the temperature of hydrolysis (37°C.). Various emulsifying agents which include trisodium phosphate and gum acacia were tried, but none proved satisfactory. Since the emulsion broke up in a short interval after emulsification, the following procedure was adopted to ensure that the fat did not float during hydrolysis. Weighed amounts of fat to be studied were melted and thoroughly mixed in excess of glycerol, vigorously shaken till the mixture cooled down to the room temperature. This process ensures a fine suspension of the fat in glycerol and does not break up for more than four hours.

Pancreatic lipase was prepared from pig pancreas by the method of Willstatter and Waldeschmidt Letz.

Table 3 : Rate of hydrolysis of fats

No.	Nature of the fat	m.p., °C.	Acidity	Peroxide value	Iodine value	c.c. of N/10 alkali required at regular time intervals (in hr.)						
						½	1	1½	2	2½	3	3½
1.	Cow ghee ..	33	0.1	4	35	1.4	3.3	5.9	7.8	9.2	10.2	10.6
2.	Refined groundnut oil ..		0.2	4	93	0.8	2.0	3.6	4.8	5.6	6.5	6.6
3.	Straight hardened oil	60	0.35	3	..	..	0.6	1.3	1.8	2.3	2.9	3.0
4.	Straight hardened oil	55	0.3	3	17	0.1	0.6	1.2	1.8	2.4	3.0	3.0
5.	Blends from 3 and 2	55	0.25	3	18	0.1	0.7	1.2	1.8	2.5	3.6	3.0
6.	Straight hardened oil	47	0.35	3	..	0.1	0.8	1.6	2.0	3.0	4.2	..
7.	Blend from 3 and 2 ..	47	0.25	4	..	0.2	0.9	1.6	2.1	3.0	4.3	4.5
8.	Blend from 4 and 2 ..	47	0.2	4	..	0.1	0.8	1.6	2.6	3.9	4.2	4.3
9.	Straight hardened oil	42	0.4	5	..	0.4	1.6	2.8	3.7	4.4	5.0	5.2
10.	Straight hardened oil	41	0.3	4	..	0.5	1.6	2.8	3.8	4.5	5.0	5.15
11.	Blend from 3 and 2 ..	40	0.3	2.5	44	0.6	1.8	2.9	4.0	4.6	5.2	..
12.	Blend from 4 and 2 ..	40	0.5	2.5	46	0.5	1.7	2.8	3.9	4.6	5.2	..
13.	Blend from 7 and 2 ..	40	0.3	3	55	0.8	1.6	2.9	3.8	4.8	5.4	5.5
14.	Blend from 9 and 2 ..	40	0.2	4	55	0.7	1.8	2.8	4.0	4.3	5.4	5.6
15.	V. O. P. Nandi ..	39	0.3	6	60	0.6	1.7	3.0	4.2	4.9	5.6	..
16.	Straight hardened oil	37	0.25	2	63	0.5	1.7	3.2	4.5	5.1	5.8	..
17.	Blend from 3 and 2 ..	37	0.15	4	65	0.7	1.9	3.4	4.8	5.3	6.3	..
18.	Blend from 4 and 2 ..	37	0.2	4	63	0.7	1.9	3.3	4.6	5.2	6.1	..
19.	Blend from 7 and 2 ..	37	0.25	3	66	0.5	1.7	3.1	4.3	5.0	5.8	..
20.	Blend from 9 and 2 ..	37	0.35	2.5	64	0.6	1.7	3.1	4.4	5.0	5.7	..
21.	Ghee, commercial (presumably adulterated)	42	3	5	..	0.6	1.2	2.1	3.3	3.9	4.6	5.0
22.	do. ..	45	2.	..	..	0.4	1.5	2.4	3.0	3.5	4.0	..
23.	do. ..	46	5	..	..	0.5	1.4	1.8	2.4	3.8	4.3	4.5
24.	do. ..	46	4.5	..	..	0.7	1.4	2.1	2.8	3.2	4.2	4.4

A 5% aqueous solution of sodium taurocholate was used as an emulsifying agent.

Ammonia-ammonium chloride buffer was used for buffering at *p*H 8.9.

A series of conical flasks was set up each containing 1 g. of fat finely suspended in 10cc. of glycerine, as describe above; 2 cc. of sodium taurocholate solution, 2 c.c. of enzyme extract and 2 c.c. of buffer solution were then added. The flasks were then kept at 37°C. with continuous shaking and removed at regular intervals of half an hour. 25 cc. of 95% hot alcohol were poured to destroy the enzyme and the contents treated against N/10 sodium hydroxide using phenolphthalein as an indicator. The titre value for the blank was taken as the zero titre.

Hydrolysis for each sample was repeated thrice and only concordant values are recorded in Table 3. Iodine values for the blended samples were determined by Wij's method.

Discussion

The various fats, straight hardened and blended, follow the following order of decreasing rate of hydrolysis : ghee, refined oil, blended vanaspati, straight hardened vanaspati and high melting hydrogenated fats.

The rate of hydrolysis of low grade ghee is very poor when compared to pure ghee and it compared very well with the blended vanaspti samples.

Blended samples of vanaspati show a slightly higher rate of hydrolysis than straight hardened vanaspati of the same melting point (a blend containing a greater proportion of the oil has a higher rate of hydrolysis).

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Studies on the nutritive value of blended vanaspati

INFLUENCE OF THE DIETARY FATS ON THE BODY AND LIVER LIPIDS IN THE RAT*

Though the fats of animals have been shown to originate in part by synthesis from dietary carbohydrate[15] and protein[16], the dietary fat is generally considered to exert a marked effect on composition.

The selective utilisation of fatty acids is considered a ' species characteristic '. Rat is one of the animals which is very responsive to the dietary fat, its body lipids being changed profoundly with the diet without apparent effect on their metabolism and general health [1–4]. The preference of certain acids for deposition is determined by the chemical and physical properties of the dietary fat associated with the chain length, unsaturation and melting point[4–11].

Ellis and Isbell[2] reported a close parallelism between the iodine value of the body fat and the food fat. Datta [12] has also shown that the body fat as indicated by the iodine value is modified in the body to resemble the ingested fat. In the above experiments, the fat furnished 60 per cent and 40 per cent of the total calorie intake which amounts to 40 per cent and 20 per cent of fat in the diet respectively. Normally fat is not consumed at such high levels. In our experiments two levels of fat have been studied which correspond to 10 per cent and 30 per cent in the diet, providing 20·5 per cent and 50·5 per cent. of the total calories respectively.

In the world, a great variety of oils and hydrogenated fats are consumed depending upon the availability and the taste of the particular oil. The fats most commonly used in India are ghee (clarified butter fat), coconut oil, groundnut oil and vanaspati (shortening).

Extensive studies have been carried out both in this country and elsewhere on the nutritive value of vanaspati. Such investigations have been invariably carried out with the straight hardened product obtained from reputed firms. As the blended product is also known to be produced, it is naturally of interest to know how it compares with the other product. These experiments were undertaken to determine the influence of partial hydrogenation and blending on the body and liver lipids as indicated by the iodine value and the percentage saturation of the total fatty acids.

Experimental

White young male rats weighing 40-50 g. each, were selected from the stock colony and divided into 18 comparable groups of six each. The fats studied were, ghee, coconut oil, refined groundnut oil, partially hydrogenated groundnut oils melting at 38°C., 45°C. and 51°C. and blends

* The work described in this section was carried out by Shri M.R. Sahasrabudhe, Central Food Technological Institute, Mysore, under the supervision of Dr. V. Subrahmanyan.
Shri M.V. Lakshminarayana Rao rendered valuable help and gave suggestions.

(containing a portion of almost completely saturated oil melting at 60°C.,) melting at 38°C., 45°C. and 51°C. respectively. Each of the 9 fats was fed at two levels (10 per cent. and 30 per cent, in the diets A and B respectively). Protein formed 20 per cent of the diet. Composition of diets A and B is given in Table 1.

Table 1 : Composition of diets

	Diet 'A'	Diet 'B'
Casein (N×6.25) extracted (g.)	20	20
Sugar (g.)	5	5
Fat (g.)	10	30
Salt mixture (g.)	4	4
Starch (g.)	61	41
Calories per 100 g. diet	434	534
°/₀Calories supplied by the fat	20.5	50.5

Table 2; Average weekly increase in body weight (g.)

	Diet 'A'	Diet 'B'
Ghee	11.58	12.60
Coconut oil	10.25	11.00
Groundnut oil	11.33	12.60
Hydrogenated oil, m.p., 38°C.	11.16	11.70
Blended oil, m.p., 38°C.	10.83	11.50
Hydrogenated oil, m.p., 45°C.	9.16	8.70
Blended oil, m.p., 45°C.	9.50	8.50
Hydrogenated oil, m.p., 51°C.	7.33	6.04
Blended oil, m.p., 51°C.	7.87	5.09

The animals were housed in individual cages and were fed the diet and water *ad libitum*. Each rat received 0.2 g. of Brewer's yeast (Squibb), 70 mg. of thiamine, 40 mg. of riboflavin and 1 drop of diluted adexoline supplying 12 I.U. of vitamin A and 2 I. U. of vitamin D. The daily diet consumption and weekly growth rates were recorded. The average weekly increase in body weight is given in Table 2.

At the end of the experimental period of 12 weeks the animals were anaesthetized with amytal, bled through the abdominal aorta and the liver excise. The intestinal tract was then removed to avoid any inclusion of the dietary fat and the rest of the whole rat minced in a meat mincer.

Extraction of the body fat—The minced carcass was twice refluxed with alcohol and exhaustively extracted with ether till all the fat was removed. The ether and alcohol extracts were distilled and the combined residue redissolved in ether, evaporated, dried in a desiccator and weighed.

Aliquots were taken for the iodine value determination and the remainder saponified and the fatty acids separated into solid and liquid fractions by the modified method of Twitchell. Iodine value was determined by the modified procedure of Kuhnhenn[14].

Liver fat—The whole liver was dried at 90°C., powdered, exhaustively extracted with alcohol and ether mixture (2 : 1) and the lipids obtained after distillation of the solvents were redissolved in petroleum ether, evaporated on a water bath and dried in a desiccator. The lipids so obtained were analysed for the iodine value and the percentage saturation in the same manner as was the body fat.

The analyses of body fat and liver fat are given in Tables 3 and 4 respectively.

Table 3 : Analysis of body fat

Fat ingested	Iodine value of the food	Percentage saturation of fatty acids	Diet 'A' I total body fat, g./100 g. body wt.	Diet 'A' II Iodine value of body fat	Diet 'A' III Saturated fatty acids, % of total	Diet 'B' I Total body fat, g./100 g. body wt.	Diet 'B' II Iodine value of body fat	Diet 'B' III Saturated fatty acids, % of total
Butter fat	38.6	51.1	15.15±1.01	60.3±1.06	28.67±0.326	20.62±1.12	56.9±1.68	35.1±1.101
Coconut oil	9.8	84.6	14.68±0.96	52.8±0.985	30.14±1.151	18.28±0.932	48.5±1.00	36.5±1.101
Refined groundnut oil ..	93.2	18.0	17.25±0.65	68.2±0.675	28.49±0.690	20.45±1.101	76.6±0.671	23.8±0.625
Hydrogenated oil, m.p., 38°C.	66.4	32.1	17.28±1.11	62.3±1.00	29.68±0.752	20.58±0.875	70.18±1.01	30.41±0.421
Blended oil, m.p., 38°C. ..	68.3	30.6	16.32±0.285	60.12±1.11	28.92±1.16	19.15±0.689	68.3±0.921	28.5±0.089
Hydrogenated oil, m.p., 45°C.	49.3	40.1	15.28±0.981	58.9±0.589	27.86±1.31	17.19±1.48	53.1±0.671	33.6±0.725
Blended oil, m.p., 45°C. ..	54.8	38.5	15.82±1.211	61.15±0.967	28.35±0.88	19.25±1.62	58.3±0.983	36.2±2.00
Hydrogenated oil, m.p., 51°C.	30.6	51.3	14.26±1.19	60.72±1.10	26.83±0.462	18.1±1.38	50.6±0.66	37.1±0.98
Blended oil, m.p., 51°C. ..	30.2	46.4	16.12±1.20	60.19±1.31	28.29±0.499	20.2±0.465	54.1±0.851	36.2±1.80

Table 4 : Analysis of liver fat

Fat ingested	Diet 'A'			Diet 'B'		
	Total liver lipids, g./100 g. tissue	Iodine value, liver fat	Saturated fatty acids, % total fat	Total liver lipids, g./100 g. tissue	Iodine value, liver fat	Saturated fatty acids, % total fat
Butter fat	4.86±0.15	103.4±1.2	36.8±1.0	8.38±0.06	86.3±1.08	36.3±1.12
Coconut oil	3.82±0.02	99.6±0.986	35.0±1.21	5.25±0.02	78.2±1.10	38.1±1.21
Groundnut oil	4.48±0.04	100.2±1.01	30.7±1.18	6.97±0.11	92.8±0.61	28.6±0.89
Hydrogenated groundnut oil, m.p., 38°C. ..	4.68±0.17	102.1±1.25	34.7±1.26	6.52±0.18	84.3±0.86	32.7±0.91
Blended oil, m.p., 38°C...	3.92±0.30	100.7±0.88	31.9±1.20	6.36±0.01	89.6±1.21	30.2±1.21
Hydrogenated groundnut oil, m.p., 45°C. ..	4.67±0.20	100.7±0.89	30.1±0.95	5.29±0.05	82.6±1.25	30.9±1.16
Blended oil, m.p., 45°C...	5.05±0.05	102.6±1.03	36.2±0.98	6.88±0.21	87.7±0.67	33.7±0.78
Hydrogenated groundnut oil, m.p., 51°C. ..	5.69±0.03	98.3±1.60	37.1±1.16	7.37±0.072	80.5±1.16	31.6±1.06
Blended oil, m.p., 51°C...	4.98±0.10	98.6±1.18	36.1±1.35	6.82±0.15	81.4±0.99	32.5±1.01

Discussion

It will be seen from the Tables 3 and 4 that a considerably increased fat deposition occurs in the body and liver of the rat, with a corresponding alteration in the iodine value and the percentage saturation of fatty acids, when the fat level is raised from 10 per cent to 30 per cent in the diet.

With the exception of coconut oil, the iodine value of the body fat and the percentage saturation of the fatty acids of the body fat vary within narrow limits of 58.9 to 62.3 and 26.8 to 28.6 respectively, inspite of the varied nature of the dietary fats when the rats are fed diet 'A' (where the protein and fat are in adequate amounts), but the iodine value and the saturation of the fed fat is reflected to a considerable extent in the body fat when the fat in the diet is increased. This is well seen in all the groups. Similarly, the liver fat has iodine values ranging from 98.3 to 103. 4 and a saturation of 30.7 per cent to 37.1 per cent. These figures are comparable to the normal figures reported by earlier workers [2,10,11] but the nature of the liver fat is considerably affected in the high fat diets.

Previous studies on body and liver fats have always been short term experiments and always on low protein high fat diets [2,10,11,12]. The results clearly indicate that all dietary fats when fed in adequate amounts in protein rich diets are modified to a large extent before they are deposited in the liver or the adipose tissue so as to conform to the threshold limits suggested by earlier workers [1,6]. But, when an excess of fat is supplied which the body cannot cope with, then only the liver and body lipids tend to be comparable to the fed fat.

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Studies on the nutritive values of hydrogenated and blended fat

INSTITUTION FEEDING EXPERIMENTS*

Institution feeding experiments were carried out with a view to determine the deleterious effects, if any, of the hardened oil in the blends (prepared by mixing hydrogenated oil, m.p., 50° C., with refined oil).

The experimental subjects were 100 orphanage children of the Good-Shepherd Convent, Mysore, and were under study from December 1949 to September 1951.

The children were divided into three age groups: A, ages below 8 years; B, ages between 8 and 12 years; C, ages between 12 and 16 years. Majority of the children were from the B age group. Each group was further divided into three. In group A with 15 subjects, the children received ¾ oz. of refined groundnut oil to which they were accustomed. Group B and C with 35 children each were fed ¾ oz. each of the hydrogenated and blended groundnut oil, respectively. The fat was fed in measured amounts in the form of the cooking medium and the diet was distributed equally in each age-group.

Since these children were previously adapted to consuming higher quantities of fat, there was no trouble regarding the acclimatization of the children as was experienced in previous experiments.

The diet consisted of: rice, 84 parts; tur dal, 2.0 parts; ragi, 5.0 parts; green gram, 2.0 parts; fat (groundnut oil), 1.0 parts; and vegetables, 6.0 parts. Meat was very rarely included in the diet.

Chemical composition of the diet (corrected for moisture) was as follows: protein, 7.1; fat, 7.2; ash, 2.1 and rest, 83.6 per cent.

Physical examination—The examinations were carried out by three physicians who had experience and training in the detection of nutritional deficiency lesions.

The examination included inspection of the eyes, nose, throat, skin, mucous membranes, skeleton and neuromuscular reaction. Each child was examined by three physicians individually at the beginning and monthly during the experimental period. Individual charts for the symptomatic conditions, as specified by the I.C.M.R., were maintained. Height and weight were also recorded. Table 1 gives percentage abnormalities observed.

* The work described in this section was carried out by Shri M.R. Sahasrabudhe and Dr. V subrahmanyan, Central Food Technological Research Institute, Mysore.

Judging from the nutritional status of the children the following conclusions can be drawn.

(1) That the orphanage diet itself is deficient in vitamins A and B causing a greater percentage of abnormality in the skin and eyes in the control group over the pre-experimental analysis.

(2) That of the two experimental groups one receiving the hydrogenated oil seems to be slightly, though not significantly, better so far as the nutritional status is concerned.

(3) The abnormalities caused in the experimental group are more due to the vitamin deficient diet than the fat supplement.

Table 1 : Status of subjects on examination with regard to abnormalities

	Pre-experimental (100*) %	Control group (15*) %	Blended fat group (35*) %	Hydrogenated fat group (35*) %
Skin				
Pallor	12	16	12	12
Pigmentation	16	16	15	20
Seborrhea	2	..	3	3
Dryness and roughness	24	30	35	30
Hyperkeratosia	21	20	28	18
Eyes				
Dry conjunctiva	16	20	25	20
Xerosis	15	16	30	20
Bitospots	8	16	15	17
Keratomalacia	12	10	15	15
Lips				
Cheilosis	15	30	30	25
Tongues				
Paleness fissures	20	30	25	27
Constitution				
Poor	10	12	12	10
Sub-normal	20	25	22	22
Anaemic	25	30	25	20

*Figures in bracket denote number of children in each group.

Metabolism—Metabolism studies were carried out with a view to determine the digestibility of the blended fats and their influence on the retention and excretion of protein, calcium and phosphorus (usual standard methods were employed).

12 healthy children were selected for the B age group. The experimental diet was necessarily the same to which the children were accustomed.

The dietary routine was as follows :

7.00 A.M.	Light tea
8.30 A.M.	Rice porridge, without any added fat (50 g. of dried food)
1.00 P.M.	Cooked rice, with 15 g. of added fat (150 g. dried food)
7.30 P.M.	Cooked rice, with 5 g. of added fat (100 g. dried food)

The children were taught to collect the metabolic products with utmost care and were allowed a period of 10 days as reorientation, the excretion were collected for 4 days (96 hr.)

Blood samples—5 to 10 c.c. of blood from each child was taken for plasma protein, cholesterol, plasma lipids, lipid-phosphorus and liver function tests. Blood was drawn from the finger tip for haemoglobin and the cell counts. The subjects were not allowed to consume any food in the morning before the blood was drawn.

Results are given in the tables 2—8.

Table 2 : Daily food intake and excretions

Group	Daily food intake (g.)	Daily Excretion	
		As faeces (g.)	As urine (ml.)
A	300	26 (20—30)	1200 (910—1520)
B	300	24 (20—30)	1310 (1000—1610)
C	300	28 (22—33)	1090 (800—1500)

Table 3 : Comparative utilisation of the fats

Group	Average daily intake (g.)	Average daily excretion (g.)	Fat absorption (%)	Supplement fat (%)
A	20.0±0	3.0±0.89	84.2±1.14	82.4±1.62
B	20.0±0	3.2±1.00	83.9±1.10	84.3±1.08
C	20.0±0	3.8±0.67	81.2±0.82	78.6±2.1

Table 4 : Nitrogen balance

Group	Average daily intake (g.)	Average daily output			Retention (g.)
		Urine (g.)	Faeces (g.)	Total (g.)	
A	3.30	1.64±0.007	1.04±0.005	2.63±0.008	0.72±0.006
B	3.30	1.38±0.008	1.13±0.006	2.51±0.010	0.81±0.009
C	3.30	1.52±0.003	1.36±0.005	2.91±0.008	0.53±0.010

Table 5 : Plasma cholestrol and lipid P level

	Plasma cholestrol mg./100 cc.	Plasma lipid P mg./100 cc.
Pre-experimental	150—190	2.5—4.5
Control	145—198	2.6—4.3
Blended oil	170—220	3.5—4.8
Hydrogenated oil	170—240	3.4—4.6

No significant difference between the three age groups A, B and C was observed.

Table 6 : Effect of the fats on calcium metabolism

(Average of 12 children, Group B)

Fat ingested	Daily intake	Average daily output			Retention
		Urine	Faeces	Total	
	mg.	mg.	mg.	mg.	mg.
Control	208	75	128.6	203.6	+4.4
Blended oil	220	81.1	128.8	209.9	+10.5
Hydrogenated oil	208	70.0	130.6	200.6	+7.4

Table 7 : Effect of the fats on phosphorus metabolism

(Average of 12 children , Group B)

Fat ingested	Daily intake	Average daily output			Retention
		Urine	Faeces	Total	
	mg.	mg.	mg.	mg.	mg.
Control	850	560	210	770	+80
Blended oil	890	564	186	750	+140
Hydrogenated oil	840	548	192	740	+100

Table 8 : Examination of blood

Group	Haemoglobin g./100 ml.	Serum proteins g.	Albumin g.	Globulin g.
A	11.2	7.0	4.0	2.4
B	11.2	7.1	3.9	2.2
C	11.2	7.1	4.1	2.2

Absorption of straight hydrogenated and blended fats

A CHYLOMICROGRAPHIC STUDY IN CHILDREN *

The observation of chylomicrons goes back as far as 1887, but it was only in 1937 that Frazer and Stewart [1] recognised the nature of these particles as fat and realized that by observing their fluctuation in relation to food, valuable conclusions concerning the absorption, storage and mobilization of the fat might be reached.

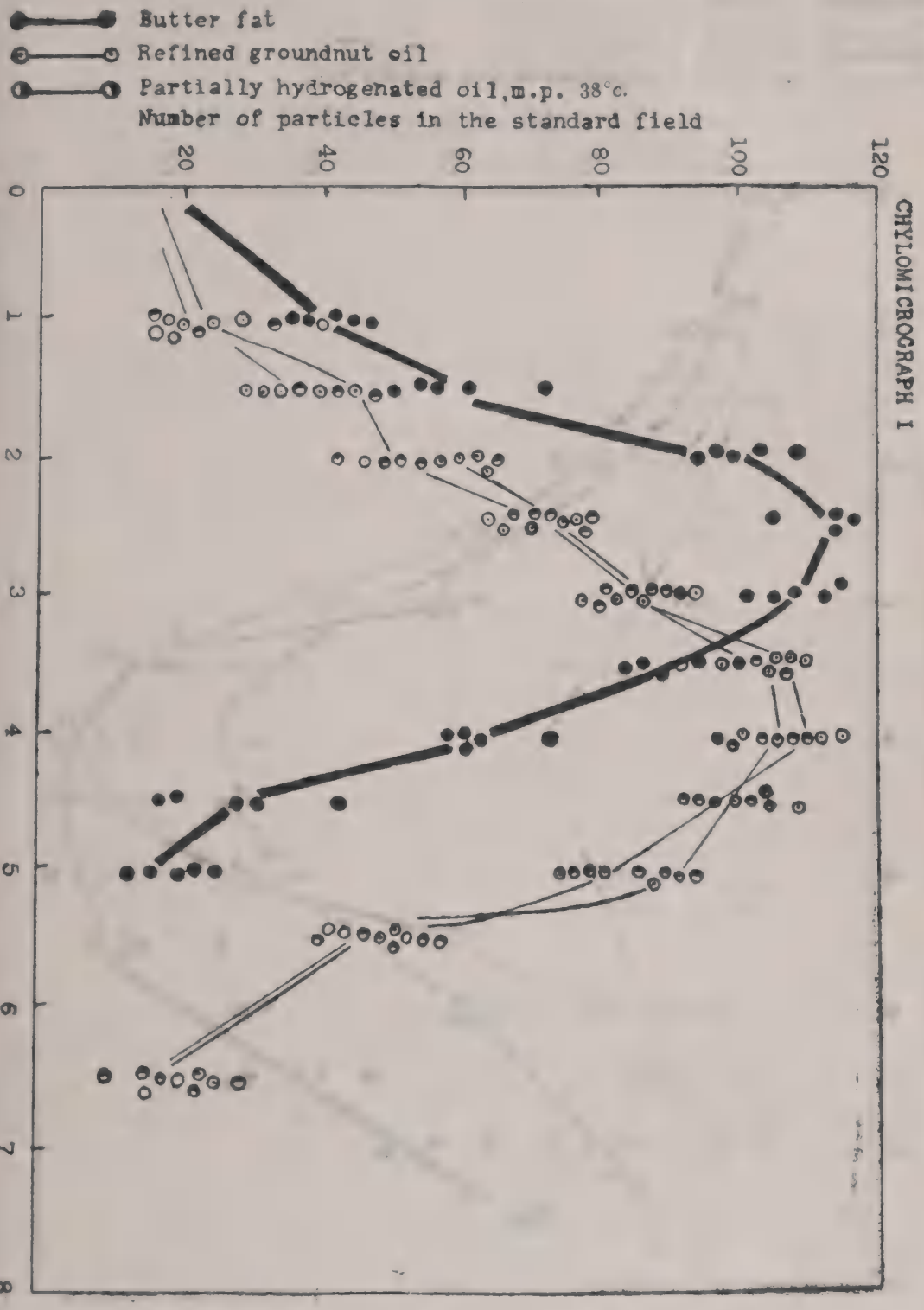
Frazer standardized a technique [2–5] for observing and counting the particles and invented the chylomicrograph—a curve indicating the number of these microscopic particles observed in blood serum under dark field illumination in a standard field, at regular intervals, after the ingestion of the test meal containing fat. It has been demonstrated that this curve is characteristic and quite constant for individual subjects, though it may vary from one subject to another. Normally, the entire elevation of chylomicron count is over in 5 to 6 hours after the standard test meal. The hyperlipaemia starts after about 1 to 2 hours after ingestion of the fat, reaches a peak in 3 to 4 hours and declines in the fifth or the sixth hour.

Nhavi and Patwardhan[6] studied the absorption of butter, ghee, groundnut oil, coconut oil and sesame oil from the intestines of human subjects. They found that the absorption of butter, ghee and coconut oil was distinctly rapid compared with that of groundnut and sesame oils. Partially hydrogenated oil is reported to be absorbed almost at the same rate as groundnut oil. They suggested that the rate of absorption of fat from the intestine is determined among other things by the presence of fatty acids of low molecular weight constituting the fat.

The Frazer's technique does not determine the quantity of the fat absorbed. Chylomicrographs, for which very small amounts of blood are required, give a comparative serial picture of blood fat changes, which is often more valuable than occasional chemical analyses in the study of fat absorption [1,2,3,7,8]. For various reasons, chylomicrographs may give more accurate information about the glycerides in the blood, than the estimate of total fatty acids. The information must be considered in conjunction with the general assessment of fat absorption. If the fat absorption is 50 per cent or more and the normal systemic hyperlipaemia does not occur it may be safely concluded that the particulate absorption is defective.

Extensive studies have been carried out in our laboratories and elsewhere on the straight hydrogenated and blended fats melting at different temperatures, and it has repeatedly been shown that the digestibility coefficients of straight hydrogenated and

* The work described in this section was carried out by Shri M. R. Sahasrabudhe and Dr. V. Subrahmanyan., Central Food Technological Research Institute, Mysore.


Butter fat
Refined groundnut oil
Partially hydrogenated oil, m.p. 38°c.
Number of particles in the standard field
20
40
60
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3
4
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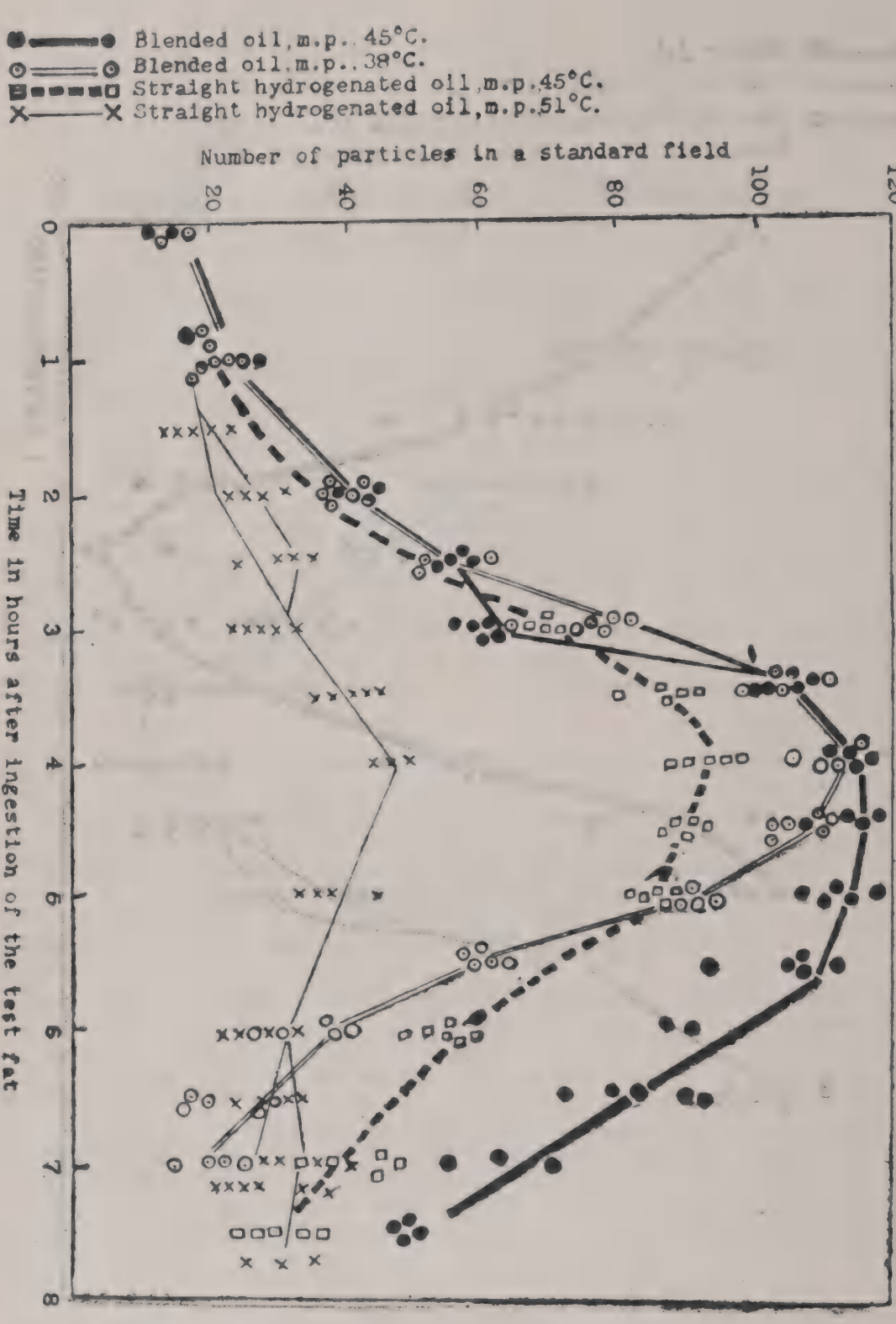
8
CHYLOMICROGRAPH I



CHYLOMICROGRAPH II

blended hydrogenated groundnut oils, melting within the physiological range are of the order of 95 : 100.

Hydrogenation, as long as the melting point of the product is within the range, has been demonstrated not to affect the rate of absorption of groundnut oil in adult human subjects [6,9]. But the influence of a completely saturated portion of the hydrogenated fat in the blend on the elevation of the chylomicron count has not been studied so far.

Experimental

The experiments reported here were designed to study the particulate absorption of blended fats and to determine whether the hardened fat portion of the blend has any marked effect on the rate of absorption. The experimental subjects were 5 normal orphanage girls between 12 and 16 years of age.These were selected from a group of 18 children after a preliminary examination of their chylomicron curves with groundnut oil. The selected children gave comparable curves. Hence, only the averages have been plotted. This preliminary examination was carried out in order to avoid as far as possible the individual variations.

Seven fats, viz., ghee, groundnut oil, hydrogenated oils melting at 38°C., 45°C. and 51°C., and blended oils melting at 38°C. and 45°C. were studied by this technique allowing at least one week after the observation on each fat, in order that the children reorientate themselves. Each observation was repeated.

The standard test meal was ¾ oz. of the test fat with 1½ oz. of bread. Other experimental conditions were essentially the same as described by Nhavi and Patwardhan (loc. cit.). The children were given the test meal at 10 A.M. after a fast of 15 hours. The first post ingestinal observation was made after 45 minutes while the subsequent observations were at half hour intervals till the seventh hour. The results are shown in the chylomicrographs I and II.

Discussion

It will be seen from the chylomicrograph that there is a considerable difference between ghee and groundnut oil and between hydrogenated and blended oils. In the first group (ghee) the entire elevation of chylomicron count was over in 5 hours after the standard meal. With groundnut oil and hydrogenated and blended oils melting at 38°C., the peak is shifted by three-fourth of an hour and reaches its original base line only after the sixth hour. With the blended oil melting at 45°C., though the peak appears almost at the same time as that for groundnut oil, the curve does not reach the basic level till after the seventh hour.

The difference between absorptions of groundnut oil and ghee has been explained by Nhavi and Patwardhan (loc. cit.) on the basis of their constituent fatty acids. They suggested that the presence of short chain fatty acids in the glyceride molecule might influence the absorption of ghee. Their results have been confirmed in our study with children. Hydro-

genated and blended fats melting at 38°C. are absorbed at the same rate as the oil.

Hydrogenated oil melting at 45°C. shows a slower rate of absorption, while with the hardened fat melting at 51°C. the chylomicron count does not rise above 50. The blend melting at 45°C. is absorbed almost at the same rate as the refined oil. The peak level being maintained at constant level for a considerable time, an accumulation in the blood of the absorbed fat is suggested. The faster rate of absorption, compared to that of the straight hydrogenated product melting at the same temperature, may be due to the presence of the refined oil forming a major portion in the blend.

There is enough evidence that the hard fats when mixed with the oil are absorbed to a greater extent. It is suggested that the hardened fats are mostly absorbed as glycerides.

Thanks are due to the authorities of the Good Shepherd Convent, Mysore, for their help in carrying out these studies on the orphanage children.

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Tocopherol in edible oils and fats *

Tocopherols are recognised as the natural antioxidants present in oils and fats responsible for the delay of rancidity. The stability of rendered animal fat has also been shown to be influenced by the tocopherol content. So it was thought highly important to study the tocopherol content of the Indian edible oils and fats. The changes produced in the tocopherol content as a result of hydrogenation and how this affects the stability was also the subject of the present study. The part that tocopherols play in nutrition as an anti-sterility and anti-dystropic factor emphasises the need for more information about the primary sources of the same in vegetable oils.

There are two methods by which the tocopherol is estimated colorimetrically. The Further-Meyar method registers all substances which give yellow-red colours with alcoholic nitric acid. The Emmerie-Engel procedure with α-α-dipyridyl registers all bodies which reduce ferric chloride. Some workers have recommended saponification before taking up the tocopherol, others recommended chromatography and another method requires both processes. The reagent of Emmerie-Engel has been widely employed for the estimation of mixture of tocopherols. Rawlings prepared the reagent with alcohol as the solvent while the Merck Laboratories developed a solvent using acetic acid. The time of interaction of tocopherols and reagent before the measurement of colour has been varied within wide limits from $2\frac{1}{2}$ min. to 10 min. The method followed in the present work is essentially that of Rawlings and co-workers with minor modifications.

Experimental

The sample of fat or oil was dissolved in dry petroleum ether (40°—60° b.p.) and diluted to such an extent that 1 cc. aliquot of the sample contains *c.* 50 to 250 μg. of tocopherol. 1 cc. of the aliquot is added to a brown glass stoppered 50 cc. bottle. 1 cc. each of 0.2% $FeCl_3.6H_2O$ and 0.5% α-α-dipyridyl solutions in absolute ethanol are added, followed by 22cc. of purified ethanol. Soon after the addition of ethanol, the timer is started. The stopper is inserted, the solution swirled gently and the bottle set aside for 10 min. A blank previously prepared with 1 cc. each of reagent, 1 cc. of petroleum ether and 22 cc. of ethanol is used to get the blank reading in the photo-electric colorimeter. At the end of 10 min., the colour developed is measured with filter No. 52.

A standard solution is prepared from standard tocopherol and the readings of the colorimeter plotted against concentration. From the graph, the tocopherol content corresponding to any reading of the unknown can be directly read off.

* The work described in this section was carried out by Shri T. A. Venkatasubramanian and B. N. Banerjee, Department of Biochemistry, Indian Institute of Science, Bangalore.

The tocopherol was estimated in some of the most commonly occurring oils and fats and the results are given in Table I.

Table 1 : Tocopherol content of some oils and fats

Oil or Fat	Tocopherol (mg./g.)
Coconut oil	0.03
Groundnut oil	0.52
Sesame oil	0.38
Cottonseed oil	0.81
Hydrogenated fat (H.V.M.*)	0.46

* Hindustan Vanaspati Manufacturing Co. Ltd., Bombay

To study the effect of hydrogenation, the sample of the raw vegetable oil as well as the hydrogenated product made from it, were analysed for tocopherol. The results are given in Table 2.

Table 2 : Tocopherol in groundnut oil

Sample	Before hydrogenation (mg./g.)	After hydrogenation (mg./g.)
1. H.V.M., Bombay	0.52	0.46
2. D.C.M., Delhi**	0.61	0.56
3. Jagdish Industries, Porbander ..	0.49	0.48
4. Tata Oil Mills, Ernakulam	0.40	0.32
5. Bangalore Vegetable Oil Products, Bangalore	0.55	0.51

** Delhi Cloth Mills, Delhi.

From the data it is found that tocopherols are not destroyed to an appreciable extent during hydrogenation. It can be safely concluded that commercial hydrogenation probably destroys little or none of the tocopherols in groundnut oil.

The antioxidant activity of sesame oil in hydrogenated fats *

It is well known that sesame oil has got good antioxidant activity when added to other vegetable oils. Previous workers have isolated sesamine and sesamoline from sesame oil, but the factor which is mainly responsible for enhancing the stability has not yet been isolated. The present work was undertaken with the idea of getting an insight into the exact role which the sterols, phosphatides, tocopherols and other minor constituents present in sesame oil contribute to the enhanced stability of other vegetable oils.

The property of sesame oil for imparting stability to other fats, when added in small quantities, has been covered by a number of patents in the literature.

In the present investigation, sesame oil was added in 5 % proportions to 2 samples of hydrogenated groundnut oil (vanaspati) m.p., 37° C. and 41° C. respectively. The keeping quality as judged by the oxygen absorption method was measured both in the case of the blank as well as the sample with added sesame oil. The peroxide value was measured for the sample after determining the induction period. The results are given in Table 1. The absorption was studied in an atmosphere of oxygen at 70° C.

Table 1 : Peroxide value of hydrogenated oil, with and without sesame oil

Sample	Induction period (hr.)	Peroxide value after induction period (In milli equivalents/kg.)
Vanaspati, m.p., 37 °C. ..	37	0.78
Vanaspati, m.p., 37 °C., +5% sesame oil	58	12
Vanaspati, m.p., 41 °C. ..	44	0.9
Vanaspati, m.p., 41 °C., +5% sesame oil	66	12.5

The sesame oil used in the experiment was freshly expressed from healthy seeds. It is interesting to note that the end of the induction period coincided with the sample developing a peroxide value of about 12 milli equivalents per kg.

* The work described in this section was carried out by Shri T. A. Venkatasubramanian and B. N. Banerjee, Department of Biochemistry, Indian Institute of Science, Bangalore.

Composition and properties of various vanaspatis and their corresponding crudes*

It was Normann's patent in 1903 that opened the way to the conversion of liquid fatty oils into solid fats. Oil hydrogenation now forms a major industry, and a variety of oils—soya bean, groundnut, cottonseed, fish oils, etc., to the tune of several lakhs of tons is being hardened all over the world, especially for edible purposes. The final product is consumed as frying fat, as shortening, in margarine, in confectionaries and as vanaspati. At one time or other, the hardened product has been considered as merely a substitute ; however, it should be really called a new product having its own special properties like plasticity, keeping quality, etc. Nevertheless, being made in a factory and produced by chemical reaction there was a good deal of opposition to its use as an edible product even in the West. We are concerned here with the opposition based on chemical considerations only and these are that hardening gives rise to some 'unnatural' components in the product, introduces a foreign element (metal) in the form of nickel and destroys some of the valuable minor constituents like phosphatides, tocopherols, etc., present in the original crude oils.

A large amount of work has already been done in the U.S.A. and Europe on the composition, constitution and properties of the various hardened products used for edible purposes. The main characteristic which distinguishes a hardened fat from the other natural fats is the presence of *iso*-oleic acid in it, the conditions which lead to its formation are now fairly well known. Similarly its effect on the plasticity, consistency and melting point of the product, is also known. Such information concerning Indian hardened oils has not been available. In the present work some of these points—especially the composition of the various vanaspatis has been investigated for the first time in a comprehensive manner. The work deals with the following aspects: (*i*) composition of various vanaspatis and their crudes in terms of oleic, linoleic, *iso*-oleic and saturated glycerides (*ii*) some chemical and physical constants (*iii*) estimation of nickel and phosphatides (*iv*) comparison of the composition of vanaspatis of certain factories produced over a period of a few weeks and (*v*) separation of *iso*-oleic acids.

The vanaspati samples and their corresponding crudes were obtained from various factories. In addition, market samples form different places were purchased for the estimation of their nickel content.

COMPARISION OF VARIOUS VANASPATIS AND THEIR CRUDES IN TERMS OF OLEIC, LINOLEIC, *ISO*-OLEIC AND SATURATED GLYCERIDES

Saturated acids in a fat can be estimated by three different methods : (*i*) Twitchel method as modified by the American Oil Chemists' Society (*ii*) Baughman-Jamieson method and (*iii*) Cocks, Christian and Harding method.

* The work described in this section was carried out by Shri G. K. Balekar and P. T. Bhide, under the guidance of Drs. J. G. Kane and K. Venkataraman, Department of Chemical Technology, University of Bombay.

The saturated acid content can also be calculated from the iodine and thiocyanogen values. All these methods, however, do not give concurrent results as will be seen from Table 1.

Table 1 : Saturated and *iso*-oleic acids in vanaspati

Sample of Vanaspati	Saturated acids			*Iso*-oleic acids		
	Twitchel method	Baughman-Jamieson method	Cocks method	Twitchell method	Baughman-Jamieson method	Cocks method
A ..	25.55	20.16	25.21	..	14.87	20.32
B ..	25.25	21.42	28.27	26.26	21.54	23.69

In the present work, the following methods were employed :

(1) Iodine value : Official and Tentative Methods of the American Oil Chemists' Society (1946, Cd 1-25)

(2) Thiocyanogen value : Official and Tentative Methods of the American Oil Chemists' Society (1946, Cd 2-38).

(3) Saturated fatty acids in unhydrogenated oils : Official and Tentative Methods of the American Oil Chemists' Society (1946, Cd 6-38).

(4) Saturated fatty acids and *iso*-oleic acids in hydrogenated fats : Cocks, Christian and Harding Method (Analyst, **56** (1931), 368).

The iodine value and thiocyanogen value of the various crude oils and their corresponding hydrogenated products, and also their olein and linolein contents are given in table 2. The two analytical constants, viz., iodine value and thiocyanogen value, for the crude oils appear to conform to the limits given in the literature. It is interesting to note that the various samples of groundnut and sesame oil show a considerable variation in their olein and linolein contents. Since these values are calculated on the basis of iodine value and thiocyanogen value only, they may require further confirmation by regular ester-fractionation analysis.

A comparison of the composition of vanaspati with that of its original oil mixture brings out certain interesting points. First is the disappearance of linoleic acid. This acid is present in the original oil mixture to the extent of 20 to 30 per cent. Excepting one, all the other vanaspati samples contain less than 10 per cent linoleic acid, the average being 5 per cent. A few samples do not contain any linoleic acid at all. Under highly selective conditions of hydrogenation, it is possible for all linoleic acid being completely converted. The practice of adding sesame oil varies from factory to factory as also the conditions of hydrogenation. If sesame oil is added to hardened groundnut oil, the final vanaspati ought to contain at least **2** per cent linoleic acid. On the other hand if a mixture of the two oils is being hydrogenated, complete disappearance of linoleic acid is quite likely.

Table 2 : Composition of vanaspatis and their corresponding crudes

Set No.	Ref. No.	Sample of oil or fat	Iodine value	Thiocyanogen value	% wt. of glycerides (*a*)			% wt. of fatty acids (*b*)	
					L	O	SG	SA	*Iso*-oleic acids
	4	GN	93.4	70.7	27.8	52.7	19.5	15.4	1.4
1	5	S	105.0	75.5	35.9	49.1	15.0	11.5	1.3
	6	V	65.9	61.6	4.9	66.7	28.4	31.5	15.1
	7	GN	93.1	78.1	18.1	71.8	10.1	15.7	0.9
2	8	S	107.9	78.4	36.2	52.6	11.2	11.0	1.1
	9	V	64.7	64.4	..	75.5	24.5	22.2	32.8
	10	GN	89.8	74.1	19.0	66.1	14.9	14.3	1.3
3	11	S	105.2	79.5	31.4	59.0	9.6	11.7	0.8
	12	CS	104.3	67.6	45.2	30.2	24.5	20.4	1.8
	13	V	65.2	65.2	..	76.6	23.4	22.9	24.0
	14	GN	93.9	76.5	21.2	66.6	12.2	18.4	0.3
4	15	S	109.8	78.5	38.5	50.1	11.4	11.7	0.9
	16	V	63.6	61.2	2.6	68.8	28.6	28.0	21.5
	17	GN	98.8	74.6	30.3	53.8	15.9	17.5	0.9
5	18	S	111.0	77.5	43.6	43.4	12.9	11.2	1.0
	19	V	70.1	63.9	7.3	66.9	25.8	24.9	25.9
	20	GN	92.6	71.1	26.3	54.7	19.0	17.1	3.8
6	21	S	104.6	75.3	36.0	49.1	14.9	12.2	3.0
	22	V	70.5	62.8	9.3	63.4	27.3	28.0	30.5
	23	GN	91.8	72.0	24.1	58.2	17.7	15.6	2.5
7	24	S	108.1	76.9	38.4	48.4	13.2	11.6	0.3
	25	V	61.2	59.0	2.4	66.3	31.3	29.2	36.6
	26	GN	93.2	72.8	24.8	58.2	17.0	16.5	0.0
	27	S	111.0	79.8	38.3	51.9	9.8	10.6	0.1
8	36	CN	8.6	7.1	1.8	6.4	91.8	34.9	0.1
	28	V	46.7	42.6	4.8	44.6	50.6	37.5	19.0
	29	GN	96.5	72.3	29.7	52.4	17.9	14.5	0.7
	30	S	111.0	79.7	38.3	51.9	9.8	11.9	0.5
9	31	CS	101.9	71.1	37.9	42.2	19.9	20.1	0.7
	32	V	69.2	63.5	6.6	67.1	26.3	23.8	37.0
	33	GN	93.9	72.8	25.8	57.2	17.0	16.1	0.6
10	34	S	108.1	77.6	37.5	50.2	12.3	12.9	0.6
	35	V	65.2	61.0	4.8	66.1	29.1	25.8	32.7
	37	GN	96.0	72.4	30.9	53.3	15.8	18.9	0.2
11	38	S	110.8	79.1	38.8	50.5	10.7	10.8	0.2
	39	V	62.2	67.3	1.9	76.6	21.5	24.4	43.9

Continued

Table 2—*Continued*

Set no.	Ref. no.	Sample of oil or fat	Iodine value	Thiocynogen value	% wt. of glycerides (*a*)			% wt. of fatty acids (*b*)	
					L	O	SG	SA	*Iso*-oleic acids
	40	GN	94.5	72.5	26.8	55.8	17.4	17.2	0.3
12	41	S	108.5	78.8	36.5	52.6	10.9	12.7	0.2
	42	V	64.8	60.9	4.4	66.4	29.2	30.3	19.2
	43	GN	93.5	75.0	22.6	63.2	14.2	15.3	0.2
13	44	S	110.3	76.7	41.3	45.2	13.5	12.9	0.2
	45	V	59.5	58.1	1.4	66.3	32.3	30.9	36.0
	46	GN	90.2	71.9	22.3	59.9	17.8	16.7	0.5
14	47	S	112.8	81.5	38.4	53.8	7.8	11.9	0.8
	48	V	64.3	61.5	3.1	68.7	28.2	26.9	31.2
	49	GN	91.8	73.2	22.7	61.1	16.2	14.7	0.3
15	50	S	105.6	77.2	34.9	53.4	11.7	12.3	0.4
	51	V	66.8	65.5	1.2	74.5	24.3	28.0	27.7
	52	GN	93.6	71.5	27.0	54.3	18.7	18.0	0.5
16	53	S	108.7	77.9	37.7	50.2	12.1	11.9	0.0
	54	V	63.7	63.1	0.4	73.4	26.2	28.4	18.8
	55	GN	92.6	73.6	23.2	61.0	15.8	16.6	0.2
17	56	S	103.8	76.1	31.0	52.3	16.7	16.5	0.4
	57	V	62.4	62.0	..	72.1	27.9	26.2	35.0
	58	GN	97.1	72.7	29.8	55.7	14.5	16.4	0.4
18	59	S	118.7	78.4	49.7	37.8	13.5	12.4	0.4
	60	V	70.2	62.3	9.4	62.7	27.9	33.3	21.3
	61	GN	88.9	71.6	21.1	61.0	17.9	17.1	0.4
19	62	S	113.8	79.3	42.4	46.9	10.7	13.1	0.3
	63	V	71.0	61.2	11.7	59.0	29.3	29.4	21.1
	64	GN	91.9	71.7	24.7	57.1	18.2	16.4	0.2
20	65	S	107.4	77.2	37.0	50.3	12.7	13.3	0.3
	66	V	71.3	65.9	6.2	70.3	23.4	26.4	20.7

Set numbers 1—20 were received from 20 different manufacturers of the country.

GN—Groundnut oil.
CN—Coconut oil.
L—Linolein.
O—Olein.
SA—Saturated acids.
S—Sesame oil.
CS—Cottonseed oil.
V—Hydrogenated product (vanaspati).
SG—Saturated glycerides.

(*a*) Iodine-thiocyanogen method.
(*b*) Lead salt-alcohol method.

If the view that all vanaspati ought to contain 5—10 per cent of linoleic acid from the nutritional aspect is accepted, it may be necessary to give a directive to the vanaspati factories to add sesame oil to the hardened groundnut oil and to carry out hydrogenation under less selective conditions. It is known that high temperature, high catalyst concentration,

poor agitation and low pressure cause selectivity. The second point concerns the *iso*-oleic acid formation. The range of its content is found to vary between 15 and 45 per cent. Under highly selective hydrogenation, more *iso*-oleic acid is produced and this is fairly well confirmed by our data, viz., high *iso*-oleic acid content goes hand in hand with low linoleic acid content. The third point is the source of *iso*-oleic acid. It is worth noting here that oleic acid is converted into its isomeric form (either *trans* form or with migration of the double bond) during hydrogenation. With regard to the saturated acids, increase in their quantity is not considerable, the average increase being about 10 per cent.

SOME CHEMICAL AND PHYSICAL CONSTANTS

In determining these constants the following methods were used :

(*i*) Acid value : Official and Tentative methods of the American Oil Chemists' Society (1946, Cd 5-40)

(*ii*) Saponification value : Official and Tentative methods of the American Oil Chemists' Society (1946, Cd 3-25).

(*iii*) Slipping point : Specification for Vanaspati, Government of India, Department of Food, notification 31st January, 1947.

Physical and chemical constants of oil and vanaspati are given in Table 3.

Table 3 : Some physical and chemical constants of oils and vanaspati

Ref. No.	Oil or vanaspati	Acid value	Sap. value	Slipping point, °C.	Ref. No.	Oil or vanaspati	Acid value	Sap. value	Slipping point, °C
4	GN	2.6	195.8	..	35	V	0.2	190.7	35.2
5	S	14.3	196.2	..	37	GN	10.8	..	..
6	V	1.0	193.1	36.2	38	S	9.8	..	..
7	GN	4.7	191.9	..	39	V	0.2	..	37.4
8	S	3.2	194.1	..	40	GN	5.0	..	..
9	V	0.3	192.2	38.0	41	S	4.8		..
10	GN	9.3	195.8		42	V	0.6	..	34.2
11	S	6.2	191.0	..	43	GN	2.7	202.1	..
12	CS	1.4	196.5	..	44	S	4.2	193.1	..
13	V	0.2	189.5	35.8	45	V	0.2	192.0	39.4
14	GN	1.4	192.4	..	46	GN	7.1	191.0	..
15	S	12.4	190.8	..	47	S	6.2	192.0	..
16	V	0.4	190.3	38.8	48	V	0.3	191.7	36.8
17	GN	1.8	202.4	..	49	GN	4.8	191.4	..
18	S	2.3	192.4	..	50	S	4.2	195.6	..
19	V	0.2	196.3	38.6	51	V	0.3	189.9	36.4
20	GN	3.8	190.5	..	52	GN	4.6	192.0	..
21	S	3.9	197.4	..	53	S	11.7	195.2	..
22	V	0.2	195.6	37.4	54	V	0.1	190.7	35.2
23	GN	4.2	196.6	..	55	GN	8.1	195.5	
24	S	5.2	197.1	..	56	S	8.4	197.9	..
25	V	0.2	191.8	39.4	57	V	0.2	192.3	37.8
26	GN	10.6	197.9	..	58	GN	2.2	195.6	..
27	S	7.6	197.6	..	59	S	2.1	189.3	..
38	CN	2.9	267.7	..	60	V	0.2	195.4	36.6
26	V	0.2	217.6	37.5	61	GN	6.0	194.2	..
29	GN	4.5	195.8	..	62	S	5.0	193.6	..
30	S	5.1	197.7	..	63	V	0.2	191.2	37.6
31	CS	2.8	199.6	..	64	GN	5.0	196.2	..
32	V	0.2	..	..	65	S	6.5	193.9	..
33	GN	4.8	191.3		66	V	0.2	193.1	38.0
34	S	4.6	190.3	..					

GN—groundnut oil ; S—sesame oil ; CS—cottonseed oil ; CN—coconut oil V—vanaspati (hydrogenated product).

The acid values of both groundnut and sesame oils (crude), varied over a wide range. Between the two, the latter, on an average, showed higher values. Cost of refining and oil losses are disproportionately large with increase in acidity and therefore the reasons for sesame oil being constantly more acidic are worth investigating. The acid values of the various vanaspatis varied between 0.1 and 1.0. Although the higher limit is legally permitted, high values indicate lack of control during processing.

The saponification values of all the crude oils varied within limits given as standard in the literature. Only one sample of groundnut oil showed an exceptionally high saponification value of 202.4 (sample No. 17).

ESTIMATION OF NICKEL AND PHOSPHATIDES

Estimation of nickel.—Since the amount of nickel present in hydrogenated fats was found to be very small, only the standard colorimetric method, using dimethyl glyoxime, was useful. Depending upon the nickel content, as was revealed by preliminary experiments, 50 g. or 100 g. of the hydrogenated fat was accurately weighed into a dish and was burnt down to a negligible residue by igniting an ashless filter paper wick fixed at the centre of the fat. The residue was ashed carefully and it was treated twice with 2 cc. of conc. HCl and a few drops of conc. HNO_3, evaporating it each time to dryness on a steam bath. 2 cc. of 6N HCl was next added followed by bromine water till the colour of bromine persisted. To remove the traces of iron present, 6N ammonia was added till alkaline, the solution was warmed, filtered and washed. The filtrate was reduced to a small volume; 1—3 cc. of alcoholic dimethyl glyoxime (1%) was added and the solution was made up to a known volume depending upon the intensity of red coloration. A reference graph was drawn for solutions containing known amounts of nickel, ranging from 2 μg. to 400 μg. per cc. of solution. The colour comparison were made by using Klett's Photoelectric colorimeter. Twenty samples of hydrogenated fats, obtained from various factories, and 18 samples purchased at different markets, were examined for their nickel contents.

The analytical figures are given in Table 4.

Table 4 : Nickel content of hydrogenated fats

Factory samples		Market samples		
Ref. No.	Nickel (p.p.m.)	Brand of Vanaspati	Market where purchased	Nickel (p.p.m.)
6	1.060	A	Bombay	0.124
9	0.160	B	Bombay	0.146
13	0.070	C	Bombay	0.660
16	2.420	D	Bombay	0.272
19	0.085	E	Delhi	0.465
22	0.370	F	Delhi	0.760
25	0.450	G	Delhi	0.340
28	0.036	H	Delhi	4.140
32	0.080	I	Delhi	2.920
35	0.050	I	Poona	7.256
39	0.420	J	Poona	1.950
42	0.370	A	Poona	0.020
45	0.130	C	Poona	0.080
48	0.035	K	Nasik	3.092
51	1.520	A	Nasik	1.680
54	0.220	A	Karad	0.650
57	0.010	L	Karad	2.100
60	0.800	M	Karad	1.260
63	0.120			
66	0.010			

On an average, the nickel content of factory samples is from 0.1 to 0.5 parts per million, while that of market samples appears definitely higher. In some cases the nickel content has gone up as high as 3—7 parts per million.

Estimation of phosphatides—Of all the methods available for the determination of phosphorus in organic compounds, the following method, which was found to be convenient for a large number of routine determinations, was adopted. Depending upon the phosphorus content of the fat, 25 g. or 50g. of the sample was weighed accurately and was ashed carefully. The ash was extracted with dil. HCl and a little quantity of HNO_3. The acidic extract was evaporated to dryness on a water bath, re-extracted with dil. H_2SO_4 and was filtered. The residue remaining on the filter was washed free of acid. To the filterate (approx. 25cc.), 5 cc. of reagent A (containing 10 g. of ammonium molybdate, 150 cc. of H_2SO_4, sp. gr., 1.84 and 200 cc. of water) was added and the mixture was immersed in a boiling water-bath for 15 minutes. Next, 5 cc. of reagent B (containing 40 g. of sodium metabisulphite, 1 g. of sodium sulphite, 0.2g. of *p*-methyl aminophenol sulphate and water to make up the volume to 200cc.) was added and the mixture was again immersed in boiling water-bath for 15 minutes. Depending upon the depth of colour, the mixture was made up to a known volume (50cc. or 100 cc.) and the colour was compared in a colorimeter with standards, containing known amounts of phosphorus. The phosphatides were expressed as lecithin after making due corrections for the blank. The analytical figures are given in table 5.

Table 5: Phosphatide content of factory samples of fats

Set No.	Groundnut oil		Sesame oil		Other oils		Vanaspati	
	Ref. No.	%P, as lecithin	Ref. No.	%P, as lecithin	Ref. No.	%P, as lecithin	Ref. No.	%P, as lecithin
1	4	0.026	5	0.033			6	0.0016
2	7	0.027	8	0.026			9	0.0008
3	10	0.003	11	0.007	12	Cotton Seed, 0.006	13	0.0003
4	14	0.001	15	0.004			16	0.0014
5	17	0.027	18	0.010			19	0.0003
6	20	0.027	21	0.020			22	0.0002
7	23	0.033	24	0.034			25	0.0054
8	26	0.011	27	0.026	36	Coconut, 0.013	28	0.0004
9	29	0.028	30	0.037	31	Cotton seed, 0.020	32	0.0026
10	33	0.028	34	0.037			35	0.0046
11	37	0.033	38	0.038			42	0.0008
12	40	0.037	41	0.031			42	0.0030
13	43	0.015	44	0.034			45	0.0064
14	46	0.014	47	0.031			48	0.0039
15	49	0.070	50	0.066			51	0.0022
16	52	0.047	53	0.062			54	0.0021
17	55	0.034	56	0.082			57	0.0010
18	58	0.078	59	0.064			60	0.0038
19	61	0.082	62	0.082			63	0.0047
20	64	0.054	65	0.011			66	0.0023

The lecithin content of the crude oils seems to range between 0.01 and 0.04 per cent. The refining procedure definitely reduces the lecithin content as is shown by the analysis of hydrogenated fats. The lecithin content of the latter ranges between 0.001 and 0.005 per cent.

VARIATION IN THE COMPOSITION OF VANASPATIS OF CERTAIN FACTORIES PRODUCED OVER A PERIOD OF FEW WEEKS

Weekly samples of vanaspati were received from selected factories over a continuous period of some weeks. The results of analyses are given in Table 6.

Table 6 : Analyses of weekly samples of vanaspati from different factories

Factory	Iodine value	Thiocyanogen value	Calculated compositions			Experimental values		Slipping point, °C.	Acid value
			Linolein	Olein	Sat. glycerides	Sat. glycerides	*Iso*-oleins		
GA	68.7	67.7	0.7	78.5	20.8	25.1	28.2	38.5	0.44
GA	68.6	66.1	2.6	74.5	22.9	26.9	34.4	38.0	0.43
GA	68.2	65.8	2.6	74.1	23.3	27.8	34.6	37.25	0.33
GA	74.9	64.6	12.4	62.1	25.5	28.6	16.9	37.0	0.34
GA	66.6	65.7	0.7	76.1	23.2	24.7	36.3	37.0	0.36
GA	67.7	65.2	2.7	73.4	23.9	25.6	33.5	37.0	0.29
GA	65.4	61.1	4.9	66.2	28.9	29.0	29.1	39.0	0.47
GA	65.5	60.9	5.2	65.6	29.2	29.5	29.3	39.5	0.47
GA	65.0	61.4	4.1	67.4	28.5	28.8	30.7	39.5	0.47
GA	65.0	61.3	4.2	67.2	28.6	29.9	29.4	39.0	0.47
GA	65.6	60.8	5.6	65.1	29.4	29.8	27.4	39.0	0.45
GA	69.8	62.1	9.1	62.6	28.2	27.9	20.5	38.0	0.41
GA	63.8	61.4	2.5	69.1	28.4	29.0	30.5	37.5	0.50
A	60.4	59.2	1.1	68.1	30.9	31.5	27.8	38.0	0.35
A	60.1	58.5	1.6	66.6	31.8	32.7	22.8	39.5	0.53
A	60.7	59.3	1.3	67.9	30.8	30.8	16.7	39.25	0.73
A	59.7	58.3	1.6	66.6	31.9	32.3	20.6	39.5	0.34
A	59.3	57.5	1.8	65.3	32.8	31.1	16.5	38.0	0.78
A	60.1	58.2	2.0	65.9	32.1	33.1	20.8	39.0	0.55
A	62.4	57.9	5.1	62.1	32.7	33.5	18.1	39.25	0.42
SW	63.5	62.4	0.9	72.0	27.1	29.5	33.6	38.0	0.14
SW	62.8	62.2	0.3	72.4	27.3	29.4	34.2	38.5	0.15
SW	61.7	59.0	3.0	65.8	31.2	32.5	34.6	39.5	0.12
SW	61.9	58.3	4.1	63.8	32.2	34.0	30.6	38.5	0.13
SW	63.3	61.0	2.5	68.6	28.9	30.5	33.3	38.35	0.25
SW	62.9	61.1	1.8	69.5	28.7	28.6	36.4	39.0	0.21
P	70.5	64.2	7.1	67.1	25.4	27.8	27.7	25.25	0.10

GA, A, SW and P are four different factories.

Out of the 27 samples received from three different sources, about six samples have shown a low *iso*-oleic content of about 16 per cent. The rest have given a fairly high *iso*-oleic acid content of about 30 per cent. Only three samples have shown appreciable quantities of linoleic acid (9-12%). The average linoleic acid content has been found to be between 2 and 3 per cent.

SEPARATION OF *ISO*-OLEIC ACID

(1) *Crystallization of hydrogenated products from solvents*—Acetone and petroleum ether were the two solvents used and the following procedure was followed.

A known quantity of hydrogenated product was dissolved in the solvent using different ratios. After cooling the mixture to the desired temperature it was allowed to remain for a definite period with occasional shaking. The mixture was then filtered through a jacketed Buchner funnel and cooled to the desired temperature with ice. The residue on the Buchner funnel was washed with a little quantity of the solvent after cooling. After determining their constants the insoluble and soluble portions were treated separately using different solvent ratios and temperature of cooling. The results are given in Table 7.

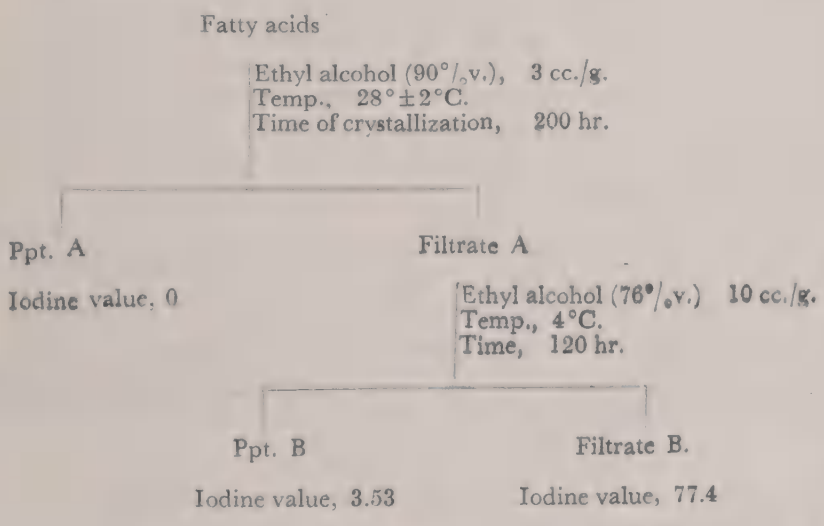
Table 7 : Crystallization of hydrogenated products from various solvents

Ref. No.	Hydrogenated product: Iodine value	Hydrogenated product: Sat. glycerides %	Hydrogenated product: *Iso*-oleins %	Solvent	Least soluble (Ppt. A): Solvent ratio c.c./g.	Least soluble (Ppt. A): Temp., °C.	Least soluble (Ppt. A): Time, hr.	Least soluble (Ppt. A): Yield %	Least soluble (Ppt. A): Iodine value	Least soluble (Ppt. A): Sat. glycerides %	Least soluble (Ppt. A): *Iso*-oleins %	Intermediate (Ppt. B): Solvent ratio c.c./g.	Intermediate (Ppt. B): Temp., °C.	Intermediate (Ppt. B): Time, hr.	Intermediate (Ppt. B): Yield %	Intermediate (Ppt. B): Iodine value	Intermediate (Ppt. B): Sat. glycerides %	Intermediate (Ppt. B): *Iso*-oleins %	Most soluble (Filtrates): Yield %	Most soluble (Filtrates): Iodine value	Most soluble (Filtrates): Sat. glycerides %	Most soluble (Filtrates): *Iso*-oleins %
35	63.9	25.8	32.7	Acetone	5	4	96	3.0	33.7	..	..	3 (Filtrate A)	4	96	33.2	49.6	..	..	63.8	71.8	..	..
45	58.7	30.9	36.0	Acetone	5	4	96	3.6	30.0	56.	26.9	..	4	96	54.8	50.8	40.9	36.6	41.6	71.1	15.4	36.0
67	63.5	29.4	33.7	*n*-Hexane (b. p., 67—71°C.)	5	4	114	24.0	40.5	51.9	34.2	..	4	114	6.8	57.1	38.4	41.8	69.2	71.4	20.7	32.7
67	63.5	29.4	33.7	,,	3	4	96	30.6	47.1	..	..	..	..	..	..	..	..	..	69.4	70.4	..	..
67	63.5	29.4	33.7	,,	2	4	96	38.4	49.1	..	..	1 (Ppt. A)	30 ±1	120	32.6	43.9	..	..	61.6	71.2	..	..

It is seen that depending upon the solvent and its ratio, the yield of the most insoluble fraction has varied form 3 to 38 per cent. In the case of very low yields one might have expected to isolate a trisaturated glyceride. However, this fraction was found to have an iodine value of about 30 and *iso*-oleic acid content of 25 per cent. Analysis of the other two portions indicates that *iso*-oleic acid is evenly distributed in all the fractions. From the content of the saturated acids in the three portions it would appear that the least soluble fraction consists mostly of disaturated monounsaturated glycerides, the unsaturation being largly due to *iso*-oleic acid. The intermediate fraction consists mostly of monosaturated glycerides and the soluble fraction consists of dioleo-mono-*iso*-olein, as a major component.

(2) *Segregation of iso-oleic acid*—Both physical and chemical methods can be thought of for the purpose of segregation *iso*-oleic acid. In the case of this acid which differs from the normal oleic acid either in its geometrical configuration or in the position of its double bond, a physical method under mild conditions is preferable since a chemical method and use of high temperatures are liable to bring about structural changes. The physical methods, on the other hand, hinder sharp separations on account of such factors as mutual solubility, association, etc. However, a systematic study of these methods has been undertaken in the present investigation to find out their applicability. All the experiments are still in an experimental stage.

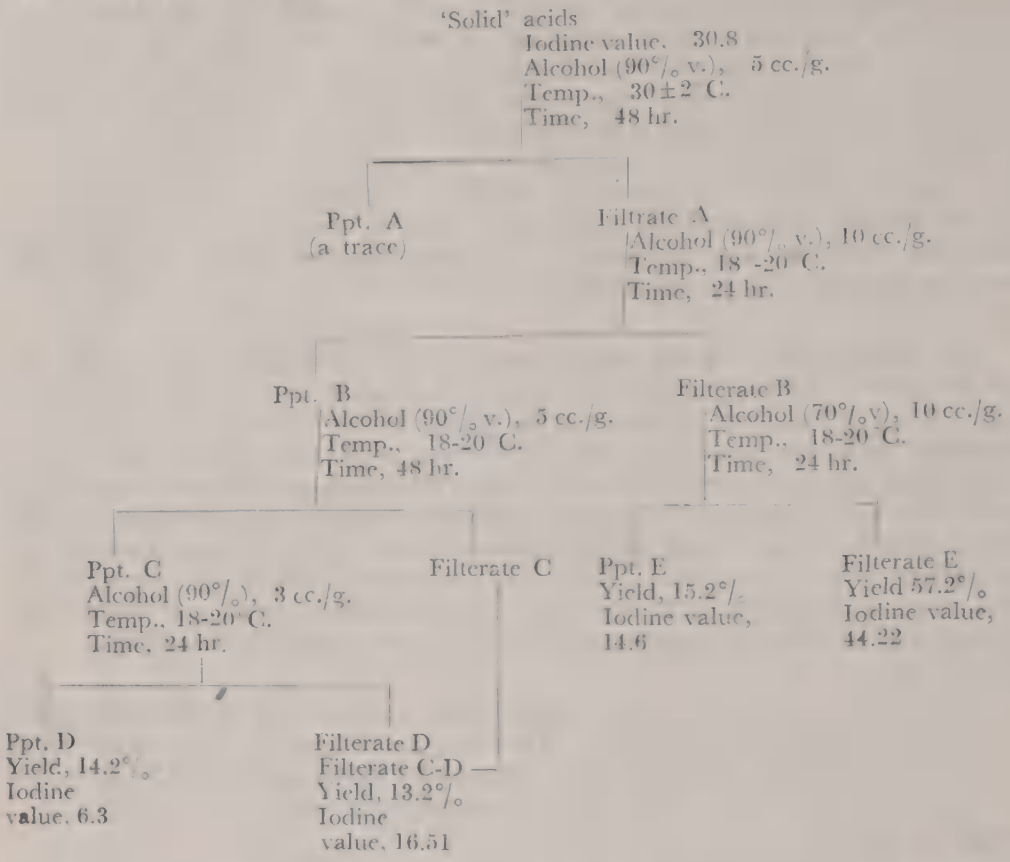
Crystallizations from ethyl alcohol were carried out using mixed fatty acids of hydrogenated products. The fatty acids used in this experiment had the following composition : Saturated fatty acids, 31.17 ; *iso*-oleic acids, 33.58 ; and oleic and other unsaturated fatty acids, 33.25 % respectively.



It may be observed that most of the unsaturated fatty acids accumulate in the alcohol soluble portion, probably due to association and mutual solubility effect.

Moore[1] prepared a pure sample of *iso*-oleic acid from 'solid' acids by subjecting them to a prolonged crystallization from 90 per cent and 70 per cent alcohol. Similar attempts were made to separate *iso*-oleic acid from

the mixed solid acids obtained from different hydrogenated products. The scheme followed is given below :



From the crystallization experiments given above, it is clear that most of the *iso*-oleic acid remains behind in the alcohol although its concentration is only about 44 per cent. This may probably be due to the presence of a large amount of palmitic acid (nearly 40°/₀ of the total saturated fatty acids) in the hydrogenated product. Moore (loc. cit.) has clearly stated that if pure *iso*-oleic is to be separated, the original fat should be low in its palmitic acid content.

(3) *Separation of iso-oleic acid by adsorption methods*—Works of Kaufmann, Cassidy, Dutton and others have shown that if a solution of fatty acids in a suitable solvent is passed through a column of activated alumina, saturated fatty acids are absorbed in preference to the unsaturated fatty acids. Attempts were therefore made to separate *iso*-oleic acid from other saturated fatty acids present in 'solid' acids from hydrogenated product.

2.5 g. of 'solid' fatty acids containing 39.7 per cent of *iso*-oleic acid were used in the following experiments :

I. Adsorption of fatty acids on alumina using *n*-hexane
Solvent : *n*-hexane, C.P., b.p. 67-71°C.
Solvent ratio : 20 cc./g.
Adsorbent column : 30 cm. × 1.2 cm.
Rate of elution : 300 cc./hr.

The results are given in Table 8.

Table 8: Adsorption of fatty acids using *n*-hexane

Fraction	Elute/cc.	Wt./g.		Iodine value	
I	*n*-hexane, 400	0.2870	59.2		
II	,,	0.0342			
III	,,	0.0598			
IV	,,	0.0500	55.8		
V	,,	0.0190			
VI	,,				
VII	,,	0.0250			43.9
VIII	,,				
IX	$CHCl_3$, 200	0.3582		49.15	
		0.8332			
Fatty acids in the adsorbent column ..		1.1668			

II. Adsorption of fatty acids on alumina using ethyl alcohol

Solvent: Ethyl alcohol (98.5% v.)

Fatty acids: 'solid' acids containing-*iso*-oleic acid, 34.0%; saturated fatty acids, 31.2%; *n*-oleic and other acids, 34.8%.

Solvent ratio: 20 cc./g.

Adsorbent column: 20 cm. × 1.9 cm.

Rate of elution: 400 cc./hr., with 100 cc. portions of alcohol.

The results are given in Table 9.

Table 9: Adsorption of fatty acids using ethyl alcohol

Fraction	Elute/cc.	Wt./g.	Iodine value
I	100		
II	,,		
III	,,	0.0944	57.3
IV	,,		

III. Adsorption of fatty acids using acetone

Solvent: Acetone, dry C. P.

Fatty acids: As in II, 1.93 g.

Solvent ratio: 20 cc./g.

Rate of elution: 700 cc./hr.

The results are given in Table 10.

Table 10: Adsorption of fatty acids using acetone

Fraction	Elute/cc.	Wt./g.	Iodine value
I	400	0.7780	51.0
II	,,	0.8998	37.9
III	,,	0.2300	24.9
		1.9078	

REFERENCES

1 Moore, *J. Soc. Chem. Ind., Lond.*, **38** (1919), 302T.

Studies on toxicity of nickel and nutritive value of *iso*-oleic Acid.*

Investigations on the effects of feeding nickel containing diets to rats and monkeys, had shown that in both these species no toxic effects could be demonstrated, even after feeding nickel continuously for four to six months between the levels of 25 to 100 mg. nickel per 100 g. of diet[1]. It was observed that small quantities of nickel were retained in the body of the animals. The retained nickel was found to be distributed among the various tissues. An attempt was, therefore, made to study the rate of accumulation of nickel in the tissues of the rat, as also its elimination from the body after the nickel containing diets were discontinued. The effect of the protein content of the diet on the accumulation of nickel was also investigated. The results of these investigations are described below.

Accumulation of nickel— 42 young rats, 4-5 weeks old, were kept on nickel containing diet (25 mg. nickel, in the form of nickel catalyst, per 100 g. of diet). 6 animals of the same age group on nickel free diets served as control. The basal diet consisted of wheat, 58 parts; bengal gram (without husk), 20 parts; skim milk powder, 10 parts; vegetable fat, 10 parts; $Ca_3 (PO_4)_2$, 1.5 parts and NaCl, 0.5 parts. Vitamins A and D contents of the diet were 70 and 13 I. U. respectively per day per rat, given in weekly doses. On analysis, this diet was found to provide the following nutrients: fat, 11.4 g.; protein, 16.4 g.; calcium 754 mg.; phosphorus 669 mg.; and iron, 20 mg./100g.

The diet was mixed with nickel catalyst to give a concentration of nickel equal to 25 mg./100 g.

At intervals of 4, 8, 12 and 16 months respectively, 4 rats were killed every time and nickel content of tissues was determined. The results are given in Table 1.

Accumulation of nickel in tissues on low protein diets—24 young rats, 4—5 weeks old, were divided in two groups of 12 each. To one group was given a low protein diet and to the other group was given a diet with protein and nickel content as in the previous experiment. The composition of the low protein diet was as follows: wheat, 25 parts; skim milk, 10 parts; vegetable fat, 10 parts; starch (tapioca), 49.9 parts; marmite, 2.0 parts; $Ca_3 (Po_4)_2$, 1.5 parts; NaCl, 0.5 parts; $Na_3 PO_4$, 1.0 parts; and Ferric citrate, 0.1 parts. Vitamins A and D contents of the diet per day given in weekly doses, were 70 and 13 I. U. respectively.

The nutrient content of this low protein diet was as follows: fat, 11.1 g.; protein, 6.8 g.; calcium, 750 mg.; phosphorus, 680 mg.; and iron, 19 mg./100 g.

* The work described in this section was carried out by Shri S. S. Phatak and Dr. V. N. Patwardhan, Nutrition Research Laboratories, Coonoor.

Table 1 : Distribution of nickel in tissues*

	No. of animals	Bones	Liver	Kidney	Spleen	Heart	Intestine	Testes	Blood	Skin
After 4 months										
Range ..	4	..	0.24–0.35	0.35–0.76	1.0–3.3	0.76—3.1	0.39–1.1	0.43	0.61—0.91	0.0—0.1
Average ..		7.5	0.28	0.55	2.7	2.1	0.61	0.43	0.76	0.07
After 8 months										
Range ..	4	6.3—9.6	0.20—0.54	2.36—3.37	4.66—5.93	2.25—2.93	0.64–0.77	1.04—1.15	0.73—1.35	0.0—0.15
Average ..		8.4	0.36	2.82	5.36	2.57	0.71	1.1	0.91	0.09
After 12 months										
Range ..	4	7.4—10.5	0.15—0.19	1.31—3.0	2.17–3.3	2.58—3.4	0.41–0.58	0.58—0.7	0.44—0.53	*nil*
Average ..		8.63	0.17	2.0	2.77	3.0	0.52	0.64	0.50	*nil*
After 16 months										
Range ..	4	6.9—7.8	0.08—0.17	1.9–2.4	0.96—2.5	1.3—2.8	0.37—0.54	0.47–0.58	0.41–0.47	*nil*
Average ..		7.3	0.13	2.3	1.9	2.0	0.46	0.52	0.45	*nil*

* All the figures are in mg. / 100 g. of fresh tissue

The diet was mixed with nickel catalyst to give a concentration of 1 of 25 mg./100 g. of diet.

At intervals of 2, 4 and 8 months, rats were killed and the nickel content of tissues was determined. After 2 months, only 2 rats from each group were killed, but at later period, 4 animals from each group were killed for nickel determinations. The results are given in Table 2. It appears that inadequacy of protein does not contribute to an increased accumulation of nickel in the tissues. On the other hand, the general tendency appears to be that on low protein diet there is less accumulation of nickel in tissues.

Elimination of nickel from the body—After 4 months of feeding of nickel containing diet, a balance experiment was conducted to determine the intake and excretion of nickel. The rats were then transferred to nickel free diet. The excretion of nickel in urine and faeces was determined at intervals, by placing the rats in metabolic cages continuously for 4 days and collecting the urine and faeces over that period. Similar observations were taken on other batches of 4 rats each, after 8, 12 and 16 months of feeding nickel containing diet. The results are given in Table 3.

Table 3 : Excretion of nickel (in mg.) after discontinuing nickel diet*

	Starting day (immediately after stopping the nickel diet)	After 10 days	15 days	20 days	25 days	30 days	40 days
After 4 months							
Ni in urine ..	0.146	0.13	*nil*	..	..	..	..
Ni in faeces ..	0.09	*nil*	*nil*	..	..	..	..
After 8 months							
Ni in urine ..	0.165	..	0.15	..	0.062	0.036	*nil*
Ni in faeces ..	0.082	..	0.009	..	*nil*	*nil*	*nil*
After 12 months							
Ni in urine ..	0.11	0.10	..	0.05	..	*nil*	*nil*
Ni in faeces ..	0.064	0.017	..	*nil*	..	*nil*	*nil*
After 16 months							
Ni in urine ..	0.135	0.10	..	0.05	..	*nil*	*nil*
Ni in faeces ..	0.053	0.016	..	*nil*	..	*nil*	*nil*

* All the figures are for 4 days of collection and average of 4 rats.

In all the above cases, the rats were killed after the last observation and the tissues were analysed for residual nickel. The results showed that with the exception of kidney, all the other tissues examined were nickel free. Small amounts of nickel were still present in some rats as shown in Table 4.

Table 2: Distribution of nickel in tissues, on diets containing 6.2 and 16.4 per cent protein*

	No. of animals]	Bones	Liver	Kidney	Spleen	Heart	Intestine	Testes	Blood	Skin
After 2 months										
Low protein diet										
Range ..	2	2.3—2.7	0.24—0.28	0.35—0.57	*nil*	..	0.27—0.33	*nil*	..	*nil*
Average ..		2.5	0.26	0.46	*nil*	..	0.30	*nil*	0.2	*nil*
Normal protein diet										
Range ..	2	2.8—3.5	0.39—0.40	0.55—0.58	*nil*	0.47—0.91	0.58—0.65	0.49	0.36—0.42	*nil*
Average ..		3.15	0.395	0.565	*nil*	0.69	0.62	0.49	0.39	*nil*
After 4 months										
Low protein diet										
Range ..	4	3.8—4.7	0.21—0.34	0.21—0.69	0.65—2.6	0.0—1.1	0.88—0.96	0.25—0.30	0.51—0.64	*nil*
Average ..		4.15	0.28	0.43	1.66	0.62	0.92	0.28	0.57	*nil*
Normal protein diet										
Range ..	4	6.0—7.9	0.23—0.41	0.41—0.99	2.3—2.9	1.6—2.9	0.44—0.63	0.59—0.73	0.53—0.65	0.0—0.03
Average ..		6.6	0.31	0.69	2.65	2.13	0.57	0.66	0.59	0.01
After 8 months										
Low protein diet										
Range ..	4	6.0—6.6	0.22—0.27	1.3—2.1	2.4—3.5	1.7—2.4	0.49—0.69	0.49—0.83	0.57—0.60	..
Average ..		6.4	0.24	1.68	2.9	1.9	0.58	0.66	0.58	0.02
Normal protein diet										
Range ..	4	6.8—8.9	0.27—0.48	1.7—2.6	3.7—4.6	2.0—2.7	0.52—0.76	0.82—0.90	0.61—0.62	0.0—0.07
Average ..		7.9	0.41	2.2	4.33	2.35	0.61	0.86	0.615	0.043

* All the figures are in mg./100 g. of fresh tissue.

Table 4: Retention of nickel in the kidney of rats

Period (months)	No. of rats used	No. of rats showing nickel in kidney	Amount of nickel in kidney (mg./100g.)
4	4	..	..
8	4	2	0.54—0.71
12	4	3	0.09—0.16
16	4	2	0.14—0.16

It appears, therefore, that the body eliminates nickel within about 30 days after nickel is withdrawn from the diets.

Nutritive value of iso-oleic acids—The method of isolation of *iso*-oleic acids has been described in the earlier reports. Feeding experiements on young rats were carried out with diets in which *iso*-oleic acids, at 5 per cent level, formed the only source of fat. For comparison, a diet containing oleic acid at 5 per cent level was fed to the control group of animals. Observations were made on the growth rates of rats over an eight week period, digestibility of the oleic and *iso*-oleic acids during the third and seventh week of feeding and on the *iso*-oleic acids content of body fat after the termination of growth study. The experiment is described below in detail.

Growth experiment—16 young rats, 4 week old, were divided into two groups of 8 each, with equal number of males and females in each group. To one group was given *iso*-oleic acids as the source of fat and to the other was given pure oleic acid. The diet was made up as follows: skim milk (defatted), 45 parts; tapioca starch, 50 parts; and fatty acids, 5 parts.

The following vitamin supplements, in mg./100 g. of diet, were given: thiamine hydrochloride, 0.5; riboflavin, 1; choline chloride, 100; calcium pantothenate, 3; inosital, 10; folic acid, 0.2; α—tocopherol, 10; and pyridoxine hydrochloride, 0.25. Each rat received in addition 100 I. U. of vitamin A and 20 I.U. of vitamin D per week, and a mixture of 0.10 g. of linoleic and linolenic acids, prepared from linseed oil, per day.

In order to find out whether the difference in average growth shown in the two groups was significant or not, the data on weights were statistically analysed. The observations are tabulated in Table 5.

Table 5: Growth of rats on *iso*-oleic and oleic acids

Group	No. of animals	Average initial weight g.	Average final weight g.	Average difference g.	Standard error	t
First Experiment						
Iso-oleic acids	8	47.0	144.75	97.75	6.1	2.6
Oleic Acid	8	45.75	125.1	79.35	3.8	
Second Experiment						
Iso-oleic acids	8	51.6	136.7	85.1	2.7	3.4
Oleic acid	8	49.4	123.7	74.1	1.6	

Table 6 : Digestibility of *iso*-oleic and oleic acids

Particulars	Third week		Seventh week	
	Iso-oleic acid group	Oleic acid group	*Iso*-oleic acid group	Oleic acid group
Total fat intake in 8 days, g.	37.5	40.04	49.2	50.3
Weight of dry faeces, g.	41.3	44.0	50.4	51.3
Weight of fat excreted in faeces g.	3.707	2.472	4.379	2.917
Fat excreted, %	9.98	6.2	8.9	5.8
Iodine value of excreted fat	80.7	89.4	79.3	88.4
Solid fatty acids in faecal fat, %	88.72	7.7	88.9	8.2
Iodine value of solid fatty acids	76.0	1.4	72.2	2.2
Iso-oleic acids in solid fatty acids, %	84.4	1.6	80.2	2.3
Iso-oleic acids in total faecal fat excreted, %	74.9	1.2	71.3	1.9

Table 7 : Analysis of body fat

Group	No. of animals	Wt. of rat g.	Total wt. of crude fatty acid (C.F.A.) g.	C.F.A. on body weight %	Iodine value of total acids	Solid acids fraction %	Iodine value of solid acids	Apparent *Iso*-oleic acids in body fat %
			First experiment					
Iso-oleic acid group								
Range	3	155—215	21.4—31.0	13.81—14.41	69.01—70.23	31.6—35.14	19.57—22.5	7.45—7.9
Average		182.7	25.9	14.12	69.7	32.8	21.1	7.7
Oleic acid group								
Range	3	134—174	18.4—24.9	13.4—14.31	79.5—80.9	31.94—33.24	1.37—2.25	0.49—0.81
Average		150.7	20.9	13.81	80.3	32.49	1.94	0.70
			Second experiment					
Iso-oleic acid group								
Range	8	130—147	18.05—20.03	13.8—14.7	68.8—76.4	31.4—32.9	20.7—22.7	7.37—7.9
Average		136.8	19.2	14.1	72.2	32.01	21.7	7.5
Oleic acid group								
Range	8	119—141	17.0—18.98	13.2—14.3	71.4—76.4	13.2—32.8	1.1—2.1	0.38—0.73
Average		129.0	17.9	13.9	74.62	31.9	1.4	0.47

In the first experiment, in Table 5, it was observed that the growth of animal was better on *iso*-oleic acids, but the statistical analysis did not give clear cut results ; hence a repeat experiment was performed. In the latter a significant difference in favour of *iso*-oleic acids was obtained. The reasons for the observed better growth on *iso*-ioleic acids are under investigation.

Metabolic experiment—While the growth experiments were in progress, faeces of all the animal in each of the two groups were collected over a period of 8 days during the third and seventh weeks and were analysed for fat. The results are given in Table 6.

After the growth experiments were over, all the rats from each group were killed and the body fat was separated according to the method used by Barbour[2]. The crude fatty acids obtained were analysed and the results are given in Table 7.

SUMMARY.

Accumulation and elimination of nickel—When nickel was fed to albino rats at the level of 25 mg. per 100 g. ration, it accumulated in certain tissues ; the maximum accumulation occurred within eight months. Further feeding of the nickel containing diet did not cause any increase in tissue nickel. On the contrary, there was a slight decrease in tissue nickel content after eight months.

On low protein diets the storage of nickel in tissues was slightly less than when the protein content of diets was within the normal range.

The body was freed of accumulated nickel within about 30 days after the discontinuation of nickel feeding.

Iso-oleic acids—In young albino rats, kept on an adequate diet with 5 per cent *iso*-oleic acids as the source of fat, the growth over an eight week period was significantly higher than on a correspoding diet containing oleic acid at 5 per cent level.

The digestibilty of *iso*-oleic acids was only slightly lower than that of oleic acid.

The albino rat appeared to be capable of utilising *iso*-oleic acids as efficiently as oleic acid for its metabolic purposes. The crude fatty acids separated from the body fat of rats fed on *iso*-oleic acids alone contained about 7.6 per cent *iso*-oleic acids.

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Studies on the stability of raw, refined and hydrogenated groundnut Oils*

The problem of the stability of edible oils in a tropical country like India is of considerable importance. The oils tend to deteriorate fairly quickly at the prevailing temperatures; the difficulty increases when in emergencies, as in war, they have to be stored for relatively long periods of time and facilities for refrigeration are limited. Even slightly rancid fats are reported to have adverse physiological effects[1,2] apart from being generally unacceptable to the palate.

Considerable quantities of raw, refined and hydrogenated groundnut oils are consumed in the country. This section deals with investigations on their comparative stabilities under different conditions and covers the following studies: (*a*) relative stabilities of raw, refined and hydrogenated groundnut oils, obtained from the same source, with and without an anti-oxidant (ethyl gallate) (*b*) relative stabilities of raw neutralised groundnut oil with different initial acidities (*c*) effect of sesame oil on the keeping quality of vanaspati (*d*) comparative stabilities of vanaspati, of m.p., 37°C. and 41°C., stored at 37°C. and 45°C. (*e*) effect of carotene on the stability of vanaspati with and without ethyl gallate, and the stability of carotene itself in carotenised vanaspati and (*f*) effects of citric and tartaric acids, both singly and in mixture, on the stability of raw, refined and hydrogenated oils.

(*a*) RELATIVE STABILITIES OF RAW, REFINED AND HYDROGENATED GROUNDNUT OILS, WITH AND WITHOUT ETHYL GALLATE

Samples of raw, refined and hydrogenated oils (m.p., 37° and 41°C.), obtained from the same source, were kept with and without ethyl gallate (0.005%) at 37°C. in sealed but not evacuated tin cans in 100 g. portions. Stability was measured by taking the peroxide, iodine and acid values periodically ; the Kreis test was applied from time to time to detect rancidity. The results are summarised in Table I.

Table 1: Comparative stability of raw, refined and hydrogenated oils Stored at 37°C.

Groundnut oil	Peroxide value			Iodine number			Acid value		
	Week of storage			Week of storage			Week of storage		
	2nd	10th	26th	1st	9th	25th	3rd	12th	30th
Raw	2.2	82.8	210.0	89.7	83.1	74.2	2.00	4.02	7.80
Raw +E.G.	2.0	45.7	87.7	89.7	87.8	76.0	2.00	2.41	5.10
Refined	1.8	84.1	218.0	86.5	79.0	69.4	0.13	2.40	5.30
Refined +E.G.	1.6	20.2	61.2	86.5	83.6	71.4	0.12	0.80	2.80
Hydrogenated (m.p., 37°C.)	0.2	76.5	181.0	66.6	65.4	64.2	0.11	1.84	3.80
Hydrogenated (m.p., 37°C.) +E.G.	0.2	15.4	41.4	66.6	66.5	66.4	0.10	0.90	2.00
Hydrogenated (m.p., 41°C.)	0.1	6.9	12.7	66.3	66.2	66.1	0.09	0.19	0.40
Hydrogenated (m.p., 41°C.) +E.G.	0.1	0.4	0.7	66.3	66.3	66.2	0.08	0.08	0.10

E.G. —Ethyl gallate

* The work described in this section was carried out by Shri B. R. Roy and Dr. B. C. Guha, University College of Science and Technology, Calcutta.

Though the refined oil appeared to be slightly more prone to deterioration than the raw oil, the stabilising action of ethyl gallate seemed to be more pronounced with the refined oil than with the raw oil. Hydrogenated oils were, as might be expected, more stable than the unhydrogenated oils. Of the two hydrogenated oils used, the one of m.p. 41°C. was considerably more stable than the one of m.p., 37°C. Ethyl gallate enhanced the keeping quality of all the samples of raw, refined and hydrogenated oils. In coming to the above conclusions, importance has been given to the percentage change in peroxide and iodine values as the measure of stability and not so much to the change in acid values. So far as the acid values are concerned, it will be seen that the percentage increase in acidity is the highest with the hydrogenated oils, although the absolute values of acidity are still the lowest for the hydrogenated oils.

(*b*) RELATIVE STABILITY OF RAW NEUTRALISED GROUNDNUT OIL WITH DIFFERENT INITIAL ACIDITIES

Raw groundnut oil samples having different initial acidities were neutralised and then compared for the keeping qualities at 37°C. The object was to see whether neutralisation would even out differences regarding the stability of these oils. Experiments were carried out with and without ethyl gallate. Summarised results are shown in Table 2. Iodine and acid values are omitted from the table, as the changes in these values are less striking than those in the peroxide value.

Table 2: Stability of neutralised groundnut oils with different initial acidities

No.	Initial acid value	Samples	Peroxide value Week of storage		
			1st	16th	24th
1	0.01	1	5.2	51.9	186.9
		1 + E.G.	5.2	8.2	11.9
2	1.35	2	10.9	169.8	242.8
		2 + E.G.	10.9	13.7	15.3
3	5.52	3	11.3	395.6	315.3
		3 + E.G.	11.3	19.4	27.2
4	10.07	4	8.9	423.8	246.7
		4 + E.G.	8.9	12.1	25.4

It was found that the rate of deterioration was the greatest with the oil having the highest initial acidity and the lowest with the one having the lowest initial acidity, although the acidity in all samples was neutralised before starting the stability test. In the case of very high peroxide values, they seemed to reach a maximum and then fall, apparently owing to further break-down of the peroxides; this has also been observed by others [3].

These observations seemed to indicate that, along with or as a result of the initial acid formation in the oil, certain reactions were set in motion or products formed which continued to influence the deterioration of the oil even after the acid itself was neutralised. Hence, for keeping quality, oils having low initial acid values are to be preferred. Ethyl gallate improved the stability of all samples strikingly.

(c) EFFECT OF SESAME OIL ON THE KEEPING QUALITY OF VANASPATI

(*i*) Refined sesame oil is a compulsory addendum to vanaspati now, as the presence of vanaspati in adulterated ghee can then be readily detected by the Baudouin test given by sesame oil. The effect of refined sesame oil on the stability of vanaspati was therefore studied. The results are given in Table 3.

Table 3 : Stability of vanaspati mixed with sesame oil (5%)

Vanaspati	Addenda	Peroxide value Months of storage		
		0	4	8
A (m.p., 37 °C.)	(*a*) Nil	1.8	4.1	6.3
	(*b*) Sesame oil	1.8	6.8	8.3
	(*c*) Sesame oil+E.G.	1.8	3.8	5.2
B (m.p., 41 °C.)	(*a*) Nil	2.4	4.7	5.8
	(*b*) Sesame oil	2.4	5.7	6.0
	(*c*) Sesame oil+E.G.	2.4	2.5	3.4

A and B are two different brands of vanaspati

Sesame oil admixed with vanaspati to the extent of 5 per cent appeared to accelerate the deterioration slightly, but with a small amount of ethyl gallate (0.005%) the keeping quality improved and the sample kept better than the original.

(*ii*) Hydrogenated sesame oil (m.p., 19 °C.) when substituted for unhydrogenated sesame oil for admixture with hydrogenated groundnut oil, afforded better protection to vanaspati, as is evident from Table 4.

Table 4 : Stability of vanaspati mixed with hydrogenated sesame oil (m.p., 19 °C.)

Vanaspati, m.p., 37 °C., containing	Peroxide value Months of storage		
	0	3	9
Unhydrogenated sesame oil	0.5	19.4	56.1
Unhydrogenate dsesame oil+E.G.	0.5	11.1	31.2
Hydrogenated sesame oil	0.4	16.2	41.3
Hydrogenated sesame oil+E.G.	0.4	4.5	22.7

Incidentally, it has been observed that sesamol, which is responsible for the Baudouin test given by sesame oil, has a stabilising effect on vanaspati; as vanaspati mixed with sesame oil from which sesamol is removed, by passing through a column of activated alumina, has a much lower keeping quality than vanaspati mixed with raw sesame oil.

(*d*) COMPARATIVE STABILITIES OF VANASPATI OF M.P., 37 °C. AND 41 °C.

As from the stand-point of digestibility, vanaspati of the lower melting point is recommended, this experiment was carried out to find whether vanaspati samples having m.p. 37 °C. would be less stable than those of m.p. 41 °C. The stability was compared at a storage temperature of 45 °C. when both would be in the liquid state. The results are given in Table 5.

Table 5 : Comparison of vanaspati of m.p., 37 C. and 41 C. stored at 45 °C.

Vanaspati	Peroxide value Months of storage		
	0	1	3
m.p., 37 °C.	0.0	3.4	60.3
m.p., 37°C+E.G.	0.0	0.0	20.5
m.p., 41 °C.	0.1	0.6	9.9
m.p., 41°C+E.G.	0.1	0.1	0.5

The results indicate that under these conditions, vanaspati of m.p., 41°C. was more stable than the other. Ethyl gallate improved the keeping quality in both the cases. Fig. 1 shows these results strikingly.

(*e*) EFFECT OF CAROTENE ON THE STABILITY OF VANASPATI AND THE STABILITY OF CAROTENE ITSELF IN CAROTENISED VANASPATI

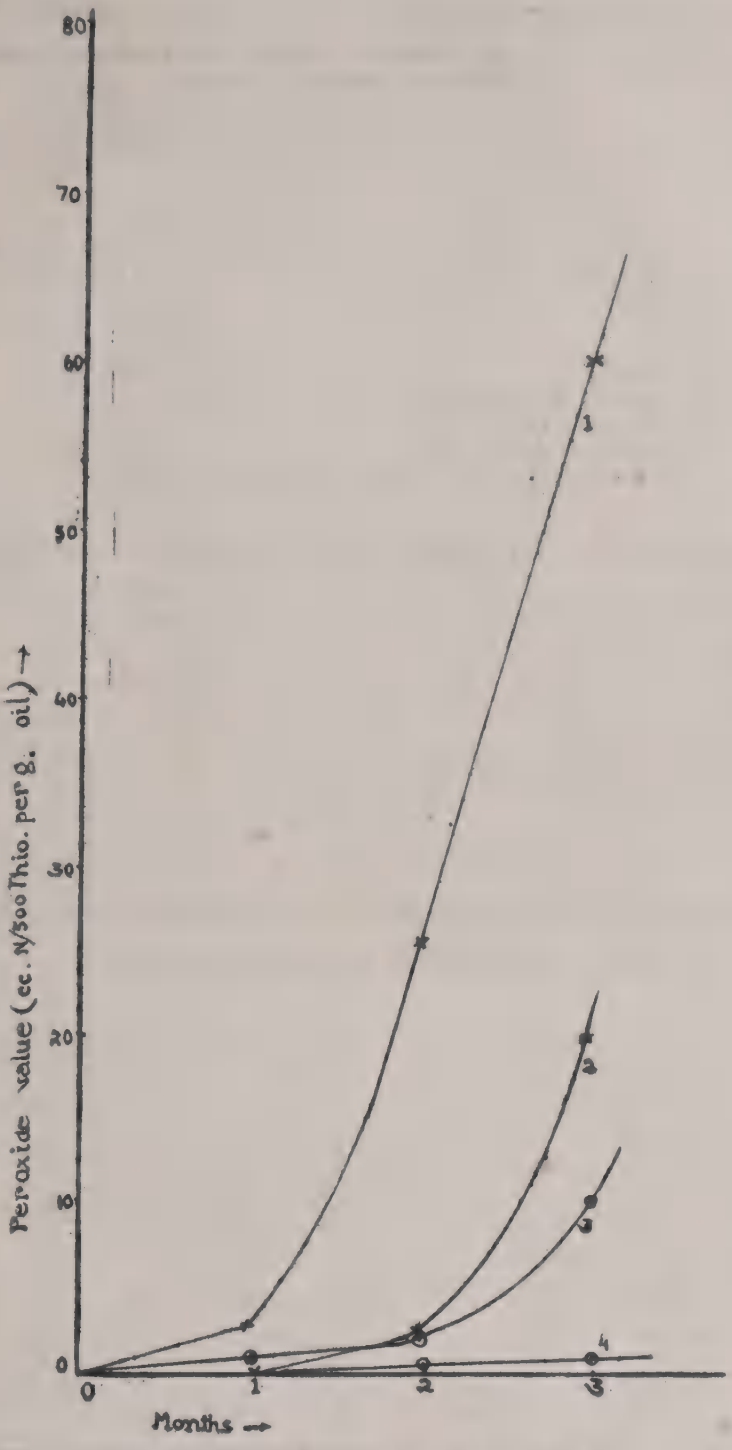
In view of the facts that consumption of hydrogenated oils does not interfere with the conversion of carotene into vitamin A [4] and that shark and other fish liver oils as sources of vitamin A might be unacceptable to many people in India, it has sometimes been suggested that certain quantities of vanaspati might be enriched with carotene which might be used for cooking not involving frying. Such fortification, however, necessitates attention towards stabilisation, since carotene may be readily lost by oxidation under various conditions. Though some work has been done on the addition of vitamin A and carotene to edible oils and on their destruction in storage [5–9], the question of addition of carotene to vanaspati seemed to require further investigation, which is reported below.

Two brands of vanaspati, 'A' and 'B' were used for this study. These were kept at 37°C. in sealed but not evacuated tin cans in 100 g. portions.

(*i*) *Keeping quality of added carotene*—Samples containing 6 mg. per cent of carotene (90% α and 10% β) were examined for the loss of carotene on storage. It was found that about 33 per cent of carotene was lost in 3 months' storage.

(*ii*) *Effect of sesame oil on the stability of carotene in vanaspati*—Since the admixture of vanaspati with sesame oil is obligatory, the effect of sesame oil on the keeping quality of carotene admixed with vanaspati was studied. It was found that 5 per cent refined sesame oil tended to promote a slight loss of carotene during storage.

(*iii*) *Effect of ethyl gallate on the stability of carotene*—Ethyl gallate (0.005%) seemed to improve the keeping quality of carotene; 33 per cent loss without the anti-oxidant was reduced to 20 per cent loss with the anti-oxidant. Ethyl gallate also nullified the effect of sesame oil and appeared to afford protection to carotene even in presence of sesame oil. Results, showing the stability of carotene in casotenised vanaspati, are given in Table 6.



Curve 1 — Vanaspati, m.p., 37 °C.
Curve 2 — ,, ,, ,, + ethyl gallate (0.005 %)
Curve 3 — Vanaspati, m.p., 41 °C.
Curve 4 — ,, ,, ,, + ethyl gallate (0.005 %)

Fig. 1—Stability of vanaspati of m.p., 37°C. and 41°C. stored at 45°C.

Table 6 : Carotene (mg. %) in different samples of carotenised vanaspati after different periods of storage

Hydrogenated oil	Addenda	Months of storage 0	2	4	6	8
A	(*a*) Carotene	6.0	4.9	4.6	4.3	4.1
	(*b*) Carotene & sesame oil ..	6.0	4.8	4.4	3.9	3.5
	(*c*) Carotene & E.G. ..	6.0	5.6	5.1	5.0	4.7
	(*d*) Carotene, sesame oil and E.G.	6.0	5.5	4.9	4.7	4.5
B	(*a*) Carotene	6.0	4.7	4.4	4.1	4.0
	(*b*) Carotene & sesame oil ..	6.0	4.6	4.2	4.0	3.3
	(*c*) Carotene & E.G. ..	6.0	5.4	5.0	4.7	4.6
	(*d*) Carotene, sesame oil & E.G.	6.0	5.3	4.8	4.5	4.3

A and B are two different brands of vanaspati

It has been reported [10,11] that even when fresh fat containing vitamin A or carotene is fed, the vitamin may lose some potency in the digestive tract before absorption unless fortified with fat-soluble anti-oxidants. Addition of ethyl gallate to carotenised vanaspati seems to be desirable from this view-point also, apart from the consideration of stability.

(*iv*) *Protective action of carotene on vanaspati*—It is interesting to note that carotene itself afforded some protection to vanaspati. Also, the slightly harmful effect of sesame oil on the stability of vanaspati, appeared to be counteracted by the beneficial effect of carotene. The results are summarised in Table 7.

Table 7 : Protection to vanaspati by carotene

Vanaspati	Addenda	Peroxide value Months of storage 0	2	4	6	8
A	(*a*) Nil	1.8	3.0	4.1	5.5	6.3
	(*b*) Carotene	1.8	2.8	4.0	4.7	5.1
	(*c*) Sesame oil	1.8	2.3	4.9	6.8	8.3
	(*d*) Carotene & sesame oil	1.8	2.2	4.2	5.3	6.2
B	(*a*) Nil	2.4	3.7	4.7	5.2	5.8
	(*b*) Carotene	2.4	3.0	4.0	4.6	4.8
	(*c*) Sesame oil	2.4	..	5.7	5.8	6.0
	(*d*) Carotene & sesame oil	2.4	3.9	5.6	6.5	7.4

It may be mentioned that some workers found carotene to be pro-oxidant as some other pigments are, inasmuch as they absorb light to initiate oxidation [12—14]. But other workers found it to be anti-oxygenic [15,16]. Heftman [17], however, reported that carotene acted as an anti-oxidant in lower concentrations but as pro-oxidant in higher concentrations. Chevallier and co-workers [18] found that carotene acted as a pro-oxidant in light but as an anti-oxidant in the dark.

P. No.	Due Date	Return date
249	28/3/63	27/3/63
—	19.11.71	20.11.71
Pp. 291	4/6/76	25/5
—	1.6.77	10/6
—	25/6/77.	27.6.77
~~Pp. 12~~	29.7.77	29/7
	13/8/77	12/8/77
—	26.9.77	21.9.77